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The Journal of Laboratory and Clinical Medicine

VOL. II. ST. LOUIS, OCTOBER, 1916 NO. 1.

ORIGINAL ARTICLES

URIC ACID IN ITS RELATIONS TO METABOLISM*

BY STANLEY R. BENEDICT, PH.D., NEW YORK CITY.

ONE hundred and forty years ago Scheele[1] first isolated uric acid from urine and from urinary calculi. He showed that the substance was acid in properties by dissolving it in lime-water and reprecipitating it with acid. Twelve years later Pearson[2] found uric acid in the tophi of gout, thus laying the foundation of Garrod's theory of this disease which was published half a century later.

Scheele and Fourcroy[3] and others studied the chemical properties of uric acid in a rudimentary way, but the work of Liebig and Wöhler,[4] published in 1838 gives the first account of carefully conducted experiments in this field. These investigators isolated and studied practically all of the oxidation products of uric acid which are known to this day. Perhaps the most important portion of their work from the biological standpoint was the demonstration that allantoin, which had been previously isolated from the amniotic fluid of the cow, could be obtained by the oxidation of uric acid. Subsequently Moritz[5] showed that suitable oxidizing agents may convert uric acid into oxalic acid and urea, both well known biological products.

Among the numerous papers dealing with the chemistry of uric acid, that of Medicus,[6] published in 1875, is of peculiar interest. In this paper Medicus proposed for uric acid the formula which seven years later Emil Fisher showed to be correct—a beautiful example of a chemical prophecy, if we may use that term.

Uric acid is the most highly oxidized member of a group of compounds termed "purines," the most important representatives of which, adenin, guanin, hypoxanthin, xanthin, theobromin, caffein, and uric acid, are represented in Table I.

*Lecture delivered before the Harvey Society, New York, April 8, 1916.
From the Department of Chemistry, Cornell University Medical College.

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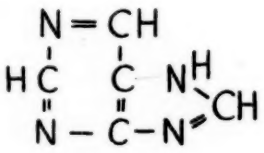
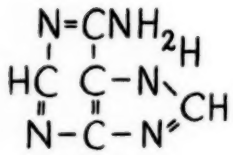
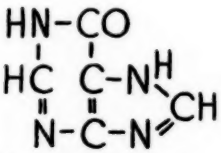


Purin



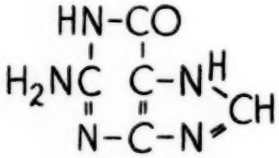
Adenin

6=aminopurin



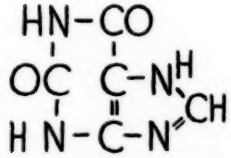
Hypoxanthin

6=oxypurin



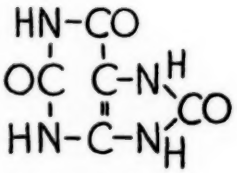
Guanin

2=amino=6=oxypurin



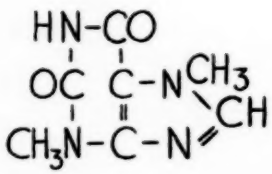
Xanthin

2=6=dioxypurin



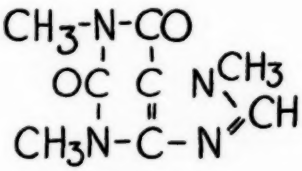
Uric Acid

2=6=8=Trioxypurin



Theobromin

3=7=dimethylxanthin



Caffein

1=3=7=trimethylxanthin

Table 1.

In coming to consider the physiology of uric acid, we may note in passing its remarkably wide distribution in the animal kingdom. It is found in nearly every animal form, from insects to man. Garrod found it in all insects examined except spiders, which excrete guanine in place of uric acid.[7] Hopkins[8] found that the powdery scales on the wings of butterflies consist of uric acid. Birds and reptiles excrete large quantities of the substance, which has also been found in the blood and urine of practically all mammals examined.

We shall now consider the substances from which uric acid may be formed in the animal body and the mechanism of such formation. In the days of Liebig the current view of the origin of uric acid was that it represented a partial oxidation product of all protein materials. If the body had a rich oxygen supply it was believed that nearly all the uric acid formed was further oxidized, while if the oxygen supply was deficient, uric acid was destroyed in much smaller proportion, and gout or similar conditions were likely to develop. This view was accepted for a long time and it was not until the early eighties that Kossel[9] laid the foundation stones of our present theories of uric acid formation. A great many men have aided in working out the problem, but we must mention especially the names of Horbaczewski, Kruger and Schmidt, Burian, Schittenhelm, Jones, and Spitzer on the biological side, and of von Baeyer, Emil Fisher and P. A. Levene on the chemical side. von Baeyer and Fisher worked out the formulæ of the purines and Levene solved the question of the structure of the nucleic acids.

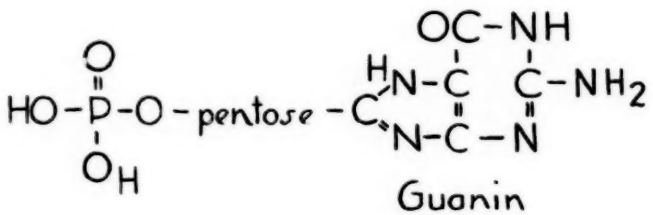
Kossel's work showed that certain purines were to be found among the decomposition products of the widely distributed nucleoproteins. Fisher had already shown the close chemical relationship of these purine bodies to uric acid and Kossel at once suggested that they might constitute the source of uric acid in the animal body. A number of experimenters, on account of unsuitable technic, both as to analytical methods and animals employed, failed to find experimental proof for Kossel's suggestion. Finally, however, Horbaczewski,[10] in a series of beautiful experiments, gave the first demonstration that animal tissues can transform the purines of nuclear material into uric acid. By digesting a tissue rich in nucleic acid, such as the spleen, with blood, he obtained the purine bases hypoxanthin and xanthin in the absence of oxygen, or uric acid when oxygen was present. Horbaczewski also found that feeding of spleen or other glands to either man or the rabbit was followed by an increase in the uric acid eliminated in the urine.

Following Horbaczewski's work, studies were undertaken by various investigators to find the mechanism by which uric acid is formed from nucleic acids in the body. In this field the researches of Schittenhelm and especially of Jones stand out preeminently.

Levene has shown that nucleic acids represent a grouping together of several simple so-called nucleotides. A typical example of a simple nucleotide is guanylic acid (Table 2) in which guanin is linked to phosphoric acid by means of a pentose radicle.

It is from the purine portion of the nucleic acids that uric acid has its chief origin in mammals. The process of the transformation is somewhat complex. The work of Schittenhelm and of Jones has shown that there are at least nine

separate enzymes which may be concerned in it. We may briefly indicate the paths for the transformation of the purines of nucleic acid into uric acid as follows: The initial splitting may be into mono-nucleotides, such as guanylic acid. An enzyme capable of effecting this splitting probably occurs in yeast and has been found in the pancreas of some species. A second type of nuclease, termed "phospho-nuclease," instead of splitting the molecule into mono-nucleotides, will split off phosphoric acid either from mono- or poly-nucleotides, leaving the purine base joined to the carbohydrate as in guanosin. A third type of enzyme splits off purine bases from the original molecule, leaving the phosphoric acid and carbohydrate radicles joined together.



Guanylic Acid

Guanin

Table 2.

So far as the purine portion of the molecule is concerned, the initial splitting by nuclease may therefore give rise to either free purine (as guanin) or to purine joined to carbohydrate, as in guanosin. The guanin is converted into uric acid through two steps. First the enzyme guanase splits off NH_2 and replaces it by an oxygen atom giving xanthin. This in turn is acted upon by xanthinoxidase which converts it into uric acid through the addition of an atom of oxygen. The transformation of the purine in guanosin may take place in either of two ways. The base may be set free by a hydrolytic enzyme and be then converted into uric acid, as we have just indicated, or the NH_2 may be replaced while the purine is still attached to carbohydrate, giving rise to xanthosin, which is then split into its two components. The xanthin is then directly oxidized to uric acid. Where the original purine is adenin instead of guanin the steps involved will be exactly analogous, with the additional step of oxidation of the hypoxanthin formed by deamidation of the adenin to xanthin.

The enzymes concerned in these transformations show widely different distribution for different species. For the most part they are found in the liver, spleen, pancreas and thymus. It is worthy of note that adenase, the enzyme which transforms adenin into hypoxanthin, cannot be demonstrated for human tissues *in vitro*. Guanase is lacking in the pig, which is subject to guanin gout.[11]

In mammals the preformed purines of nucleic acid constitute the chief, if not the only source of uric acid. In birds and reptiles this is not the case. Between 60 and 70 per cent of the total nitrogen of the urine in these species is eliminated in the form of uric acid, and it is obvious that, in such animals, there must be sources of uric acid other than the nucleic acids. Minkowski[12] has

made a special study of this problem in geese and has shown that in this species uric acid can be synthesized from the very simple compound ammonium sarcolactate. In geese with extirpated livers uric acid almost wholly disappears from the urine, its place being taken by ammonia and sarco-lactic acid. After Minkowski's work Kowalewski and Salaskin[13] furnished final corroboration of his results by showing that if blood containing ammonium lactate be perfused through an isolated goose liver it is changed into uric acid. The nitrogen of urea and of various amino acids has also been shown to be converted into uric acid under such conditions.

There is thus a two-fold source of uric acid in birds; viz., from the nucleic acids and from purine-free nitrogenous compounds. The question naturally arises as to whether there may be such a two-fold origin of uric acid in mammals.

For the young animal this question is easily answered in the affirmative. A growing animal on a milk diet, for instance, receives no nuclein or other purine-containing substance in the food, and yet eliminates uric acid and other purines in the urine and synthesizes a large amount of nuclear material for the body cells. Hence, such an animal must have the power of synthesis of purines from nonpurine materials. In the case of adult animals the question is not so readily answered. The food here constantly contains appreciable quantities of purines, and the impression has prevailed very widely that these food-purines constitute the sole source of the purine bodies catabolized in the adult mammal. At least, definite proof has been lacking that this is not the case, since the ingestion of nonpurine food substances of widely varying structure has not led to any specific increase in the uric acid eliminated. We shall return to this question later. In the meantime we may consider some of the factors which appear to influence uric acid formation, especially in man.

Burian and Schur[14] were the first to call attention to the fact that under ordinary conditions of diet there must be a double source of uric acid in the adult mammal; viz., the nuclear or purine material ingested in the food, and the nuclear material catabolized by the body from its own tissues. To the uric acid formed from the food purines Burian and Schur gave the name "exogenous" uric acid, indicating its outside origin, while the uric acid formed from the body's own store of nuclear material was termed "endogenous" uric acid. In accordance with this view it is found that when a man changes from an ordinary mixed diet to one which contains no nuclear or purine material the uric acid eliminated falls markedly in amount but does not by any means wholly disappear. That portion which does disappear is obviously exogenous in origin; that which persists is endogenous.

Studies on exogenous uric acid formation have been carried out by a number of observers. Burian and Schur, Minkowski, Kruger and Schmid, and more recently Mendel and Lyman, in an interesting and extensive investigation, have reported valuable observations upon the conversion of free purine bases into uric acid in man. Mendel and Lyman[15] found that about 60 per cent of ingested hypoxanthin, 50 per cent of xanthin, 19-30 per cent of guanin and 30-37 per cent of adenin were eliminated as uric acid.

Although uric acid elimination may be much increased after the ingestion

of glandular tissues, such as thymus, only a small fraction of the purine nitrogen reappears in the urine as uric acid. Givens and Hunter[16] have recently reported similar results following ingestion of pure sodium nucleate. What becomes of the balance of the purine nitrogen in these experiments is unknown. The suggestion that it undergoes bacterial decomposition in the intestinal tract is not very satisfactory, in view of the very high percentage which is usually lost.

We may summarize the findings concerning exogenous uric acid by saying that it seems definitely proven that purines, ingested either as free base or in the form of nucleic acid, are at least partially converted into uric acid. The possibility that the purines may have some other path of catabolism than through uric acid has not been excluded.

In a discussion of endogenous uric acid I am reminded of a statement made by Professor Mendel in his lecture before the Harvey Society ten years ago upon the subject of uric acid formation. In summarizing some problems for the future Professor Mendel said, "We assuredly need to know more about the origin and significance of endogenous uric acid." It is, perhaps, a sad commentary upon progress that after these ten years it seems necessary to alter Professor Mendel's statement and say, "We want assuredly to know *something* about the origin and significance of endogenous uric acid." The subject is almost chaos and we have neither time nor inclination to enter into a discussion of the conflicting results of various investigators. There are, however, a few facts which may bear scrutiny. The lowest possible level of uric acid excretion seems to be reached upon a diet very poor in nitrogen but which yields calories enough to protect most of the body's own tissues. Folin called attention to this fact some ten years ago and it has recently been corroborated in an interesting paper by Ringer and collaborators. If the nitrogen intake be slightly higher than the minimum level obtained with a starch and cream diet, there is a slightly greater output of uric acid. If the protein intake be again raised there is no increase in the uric acid output, at least until the nitrogen ingested is well above that contained in our ordinary diets. With a protein intake of 30 or 40 grams of nitrogen per day the uric acid elimination will again show a marked rise. This latter point is shown very beautifully in a recent paper by Taylor and Rose.[17] It is probable that the extra uric acid eliminated in such an experiment is not formed from the protein ingested but rather represents body nuclear material broken down under the influence of the forced protein feeding, a possibility suggested by Taylor and others, or perhaps the excessive activity of the digestive glands may be an important factor in such cases.

Concerning the organs or tissues which may be directly responsible for the production of uric acid, very little can be said. There are only two theories which deserve mention in this connection. One of these, suggested by Burian, assumes that the free hypoxanthin of muscle tissue is converted into uric acid and that such conversion is increased by muscular work. Burian's experiments seem to support this view, but Siven[18] was not able to reach definite conclusions as a result of his work in this connection. This latter investigator found that uric acid elimination was higher during the waking hours than during the night but was unable to demonstrate an effect of muscular work. In spite of a recent paper by Ringer[19] and his coworkers which contains a single

experiment supporting Burian's view, we must conclude that at present nothing can be definitely stated concerning a possible influence of muscular work upon uric acid elimination.

The view that glandular activity, especially that of the digestive glands, is responsible for the formation of some or all of the endogenous uric acid, was proposed many years ago by Mares and has been the subject of much work and more controversy. Siven has opposed the theory, while Hopkins and Hope,[20] Smetanka[21] and others have offered evidence in its favor. Mendel and Stehl[22] recently reported further experiments which favor the view that the digestive glands may contribute to uric acid formation. Their results, like those of Smetanka, seem to show a definite rise in uric acid elimination following an ingestion of purine-free food (protein or fat plus carbohydrate). The hourly variations in uric acid elimination during fasting are so wide in the work reported by Mendel and Stehl that one must be cautious in drawing conclusions from their experiments. The Folin-Shaffer method for uric acid determinations which they used is hardly suitable for determining *hourly* uric acid elimination. A repetition of their work employing the micro method of analysis for uric acid is urgently called for. Nevertheless, we may say tentatively that the balance of evidence is in favor of the view that the digestive glands may contribute to uric acid formation.

The urine of practically all mammals except man and the anthropoid ape contains an oxidation product of uric acid, allantoin, in considerable quantity and the uric acid is correspondingly diminished, or almost absent. Furthermore, various tissues of these species which eliminate large amounts of allantoin in the urine have been found to have the power of destroying uric acid *in vitro* with a corresponding formation of allantoin. The tissues of man and the higher apes do not exhibit this power of uric acid destruction and only minimal traces of allantoin occur in the urine of these species. Hence, it is concluded that uric acid is certainly not the chief end product of purine metabolism in any mammal so far studied except man and his first cousin. By determining the ratio of allantoin nitrogen to uric acid nitrogen plus allantoin nitrogen in the urine, Hunter and Givens have calculated what they call the "uricolytic index," or power of uric acid destruction for various species. This index shows uric acid destruction varying from 79 to 98 per cent in widely separated species of the lower mammals, while in man and the chimpanzee the index would be approximately zero.

Schittenhelm has long contended that man possesses quite a marked power of uric acid destruction in spite of the fact that Wiechowski has been able to recover in the urine nearly all of the uric acid injected subcutaneously into man and in spite of the further fact that no power of destruction of uric acid can be demonstrated in human tissues postmortem. While the evidence which Schittenhelm offers in favor of uric acid destruction by man is far from convincing and has been recently partly refuted in a paper by Fine,[23] it is also true that the evidence against this view is not final. It has been found, as will be mentioned later, that certain free purine bases behave differently in the body from the same purines when given in combination in nucleic acid, and if it should be shown that uric acid or the amino purines may go to other products

than allantoin, then Schittenhelm's view may have a certain degree of plausibility. If, for instance, uric acid, as formed in the organism were in a combination, it is possible that such uric acid might be destroyed, although free uric acid, either injected subcutaneously or mixed with tissue post mortem, might escape such destruction. As we shall see later, there are some facts which might lend support to these suggestions.

The study of uric acid metabolism has been much hampered by the fact that no animal has been available for experimental purposes in which uric acid is an end product of metabolism or in which the essential features of human purine metabolism appear to be duplicated. It is therefore of interest to know that recently an animal has been found which appears to fulfill these conditions and in which it seems probable that we shall find some of the missing links between the purine metabolism of man and other mammals.

The animal in question is the Dalmatian breed of dog. This breed is commonly known as the coach or carriage dog and is characterized by its spotted or mottled appearance. A specimen of this breed of dog encountered in the laboratory a little over a year ago showed a very peculiar anomaly of uric acid metabolism. Simple addition of hydrochloric acid to the urine of this animal was followed by the immediate formation of a heavy amorphous precipitate. On standing a short time this precipitate assumed the characteristic form of pigmented uric acid crystals. Isolation and analysis of the purified crystals showed them to consist of uric acid. The substance has repeatedly been isolated in large quantities from this urine and analyzed. An examination of the urine of this animal showed that upon a purine-free diet it was eliminating almost as much uric acid per day as would an average sized man on a similar diet, and this in spite of the fact that the dog weighs only about ten kilograms. Furthermore, uric acid injected under the skin was eliminated quantitatively as such in the urine. These findings are the more noteworthy because the ordinary dog shows a destruction of from 98 to 100 per cent of uric acid either subcutaneously introduced or that formed in metabolism. It was at first supposed that the anomaly of purine metabolism existed only in the one individual but further examination has shown that it is probably a peculiarity of the breed of Dalmatians. We have examined five animals more or less pure Dalmatians and in four of them have found a very high uric acid elimination. The one exception was obviously not of very pure breed.

It may be noted in passing that it seems difficult to find any analogy for the perversion of purine metabolism in the Dalmatian. Conditions such as cystinuria or pentosuria, which are found in some human individuals, would be closest to it, but in these cases the peculiarity is individual and not racial.

In connection with the experiments reported upon the Dalmatian dog, I desire to express my indebtedness to Mr. Emil Osterberg of our laboratory, whose constant and most efficient help has been invaluable.

Table No. 3 shows the general metabolism of our Dalmatian as represented by the urinary findings upon purine-free diets with varying nitrogen content. It will be noted that the uric acid output is not increased following a fourfold increase in the nitrogen content of the food. The allantoin (determined

by Wiekowski's method) shows, on the contrary, a distinct rise following the increased nitrogen ingestion.

TABLE No. 3.

Total N. grms.	Creatinin N. grms.	Uric Acid N. grms.	Allantoin N grms.	Remarks
4.8	0.103	0.123		
5.0	0.106	0.119	0.057	Diet contains 5.9 grms.
4.9	0.103	0.126	0.064	total N.
4.9	0.105	0.121	0.061	
5.1	0.104	0.120	0.051	
16.6	0.106	0.117	0.037	
18.4	0.110	0.119	0.107	Diet contains 24.07 grms.
19.8	0.109	0.128	0.103	total N.
17.5	0.111	0.123	0.102	
15.2	0.104	0.116	0.083	
5.4	0.104	0.154	0.073	
4.1	0.100	0.139		Diet contains 2.03 grms.
3.4	0.100	0.139	0.068	total N.
3.0	0.094	0.141	0.054	
2.8	0.091	0.139	0.059	
2.7	0.084	0.140	0.057	

For a period of nearly a year the animal has been upon a purine-free diet and during nearly all this time the uric acid elimination has been determined daily. As a result of this study we can definitely conclude that the adult mammal can synthesize purine from nonpurine material. During the period of observation the dog maintained a constant body weight and eliminated a total of more than 100 grams of uric acid. Not 10 per cent of this quantity of uric acid could have come from the preformed purine of the animal's tissues. So far as I am aware, this experiment is the first which definitely shows that an adult mammal can synthesize the purine nucleus.

The influence of caffein ingestion upon uric acid elimination is a question which has received much study. A number of investigators have failed to note any increase in uric acid output after the feeding of caffein to man.[24] Taylor[25] obtained the opposite result but the general conclusion has been that caffein ingestion in man does not lead to increased uric acid formation.

Table No. 4 records two experiments upon our Dalmatian to determine the effect of caffein given subcutaneously. A daily dose of 100 milligrams of the drug is followed by a slight decrease in the uric acid output. Although the variation is not marked, we have obtained it in each of three similar experiments. With a larger dose (200 milligrams daily) of caffein there is scarcely any perceptible effect upon the uric acid output but there is a very notable retention of nitrogen during this period.

In Table No. 5 is recorded an experiment to study the effect of caffein ingestion upon the uric acid output in man. The micro method of analysis was employed for the uric acid determination, a method which we have found to be highly accurate. The subject was placed upon a purine-free diet which was kept approximately constant but which was not weighed. During the prelim-

inary period five cups of a caffein-free coffee (Kaffee Hag) were ingested daily. During the caffein period the diet was just the same but to each of the five cups of coffee taken were added 200 milligrams of caffein, making a total of 1 gram of caffein per day. The uric acid figures of the urine showed a slight, but

TABLE No. 4.

Total N. grms.	Uric acid N. grms.	Allantoin N. grms.	Remarks.
5.37	0.129		
5.65	0.126	0.213	
6.07	0.128		
5.52	0.125		100 mgm. caffein.
5.41	0.113	0.192	" " "
5.23	0.116		" " "
5.13	0.115		
5.20	0.128		
5.34	0.127		
5.31	0.128		
4.82	0.136		200 mgm. caffein.
4.80	0.139		" " "
4.60	0.127		" " "
4.22	0.123		" " "
4.22	0.128		" " "
4.51	0.125		
4.72	0.124		
4.90	0.122		
4.96	0.127		

definite and progressive increase during the caffein period, which increase was still somewhat apparent for two days after the caffein intake was stopped. This experiment was so carefully conducted and the results were so clear cut, that I believe we are justified in concluding from it that caffein may lead to increased

TABLE No. 5.

SUBJECT E. O.

Volume C.C.	Total N grms.	Creatinin N grms.	Uric acid grms.	Remarks.
1300	12.60	0.495	0.540	
1290	13.12	0.486	0.522	
1300	13.74	0.508	0.540	
1240	10.76	0.504	**0.558**	1 grm. caffein.
1640	10.42	0.484	**0.564**	" " "
1210	8.03	0.519	**0.642**	" " "
1080	10.74	0.531	**0.702**	" " "
1200	9.05	0.543	0.636	
1100	13.53	0.531	0.600	
1400	12.51	0.502	0.546	

uric acid formation in man, and furthermore, as evidenced in Tables Nos. 3 and 4, that it may lead to some nitrogen retention. Indeed, on the basis of our experiments, we may question whether caffein, even in small doses, is quite so innocuous a substance as has been assumed.

In Table No. 6 experiments are reported showing the effect of the addition of nuclear material in the form of thymus gland to the diet of the Dalmatian. It will be noted that after the thymus ingestion the increase in the uric acid eliminated is not nearly so great as the increase of allantoin. Thus, on the purine-free diet the uric acid nitrogen is more than double that of the allantoin, while after the thymus ingestion the increase in uric acid nitrogen eliminated is only about one-half the increase to be found in the allantoin nitrogen. These results might be taken to indicate that exogenous nuclear material undergoes catabolism along different lines from that of the endogenous purine-containing material. The question involved will require further work before any definite conclusion can be drawn. This is especially evident when we note (as shown in Table No. 6) that the uric acid administered subcutaneously is followed by a marked increase in the allantoin output, and this in spite of the fact that the uric acid is recovered quantitatively as such in the urine. It seems probable that uric acid and allantoin are interrelated in metabolism in other ways than have been heretofore assumed.

TABLE NO. 6.

Total N. grms.	Urea + Ammonia N. grms.	Uric Acid N. grms.	Allantoin N. grms.	Increase in Uric Acid N. grms.	Increase in Allantoin N. grms.	Remarks.
4.37	3.86	0.120	0.050			
4.54	4.03	0.125	0.050			Normal diet.
4.82	4.36	0.127	0.050			
6.48	5.87	0.177	0.210			Same diet + 100 grms.
6.86	6.15	0.197	0.210	0.223	0.480	thymus daily =
7.30	6.58	0.200	0.210			0.32 grms. purine N.
5.75	5.27	0.129	0.058			
5.54	5.08	0.132	0.058			Normal diet.
5.27	4.84	0.137	0.058			
5.18	4.50	0.290	0.115			Same diet + 500 mgm.
5.13	4.47	0.299	0.142			of uric acid sub-
5.00	4.30	0.305	0.133			cutaneously.
5.02	4.54	0.124	0.066			

We finally turn to what may be regarded as essentially the most modern field of uric acid research. I refer here to the investigations of uric acid in blood. This field of work will be irrevocably associated with the name of Otto Folin. For many years Folin, more than any other man either here or abroad, has been a maker of high grade tools for the biochemist. In methods of uric acid research he has recorded another notable achievement. Before the Folin method was available it was questionable whether uric acid existed at all in normal human blood and quantities up to 300 cubic centimeters were necessary for even approximately accurate results with bloods known to contain an excess of uric acid. Now we are able to determine uric acid with accuracy in twenty or even in ten cubic centimeters of normal human blood. The method is equally applicable to the blood of other species.

The finding of uric acid in pathological human blood dates back to the work

of Garrod in 1848. With a truly outrageously inadequate method of analysis this brilliant worker was able to obtain results of permanent value and to demonstrate for the first time that there is an accumulation of uric acid in the blood of gout and nephritis. But from the time of Garrod until the introduction of the Folin method almost no progress was made or even attempted in this field of work.

The recent researches on uric acid in blood have yielded some results of interest in connection with lower animals as well as in man. In order to appreciate these results we must look backward for a moment. A hypothesis to explain gout, based upon the presence of two forms of uric acid in blood, was put forth a number of years ago and the questions involved here have received a great deal of attention from the experimental side ever since. Pfeiffer[26] thought that he had obtained proof of two forms of uric acid in urine but his work was shown to be erroneous. Kossel and Neumann, Goto,[27] and Minkowski called attention to the fact that uric acid seems to form a compound with thymic acid and Minkowski suggested that uric acid may circulate in the body in this form. There has been no experimental proof that this is the case. One interesting observation of Minkowski's has been interpreted as showing that some purines, at least, are catabolized in the body in combination and not as the free base. Minkowski found that when free guanin was given to dogs the gastrointestinal tract showed marked inflammation and a crystalline deposit of what he thought was adenin was to be found in the kidneys. These effects did not follow the ingestion of adenin in the form of nucleic acid. Subsequently, Nicolaier[28] showed that the deposits found in the kidneys after adenin ingestion consisted of di-oxy-adenin, thus showing that the organism may oxidize a purine before splitting off the NH_2 group. Such observations as these, and especially the more recent work of Bloch,[29] have tended to show that uric acid may exist in the organism in more than one form, but positive evidence has been lacking. Through use of the recent methods of blood analysis it has been shown beyond a doubt that in some mammals, at least, uric acid exists in the blood chiefly in combination. In fresh ox blood, for instance, using the Folin method of determination, we find about one-half of one milligram of uric acid in 100 grams of blood. If, however, the blood filtrate after removal of protein is boiled with hydrochloric acid and then the uric acid determined, it is found that the quantity present is more than 1000 per cent of the figure originally obtained. The same figure is ultimately reached if the whole blood is simply allowed to stand, thus indicating that an enzyme is present in blood which can split the uric acid combination. The combined form of uric acid is contained wholly in the corpuscles of the ox blood. In birds, in which uric acid is an end product, the blood contains none of the combined uric acid and the free uric acid present is almost wholly in the serum. It is of interest to note that the blood of the ox, an animal which eliminates almost no uric acid in the urine, contains actually 50 per cent more uric acid than does that of birds. In the ox blood it is combined and in the bird's blood it is free. These results have been received with considerable skepticism in many quarters, but since the uric acid can be readily quantitatively isolated as such, the correctness of the work is not open to question.[30] The results seem to show that it is probably form

rather than quantity of uric acid in blood which is of importance. These findings have been extended to the blood of other species and the results have shown that, with the exception of man, all mammals probably have two forms of uric acid in the blood. In the case of human blood the data so far available are not conclusive. It is quite probable that here, too, uric acid exists in the blood in at least two forms but they are quite unlike the forms present in ox blood. A new technique is being developed to study this question.

The clinical findings in regard to uric acid in human blood are of considerable interest. The field of work here is new and we must be cautious in drawing conclusions. The recent researches of Folin and his pupils in Boston and of Myers and Fine at the Post-Graduate School in New York have shown that normal human blood contains from one to three milligrams of uric acid in 100 grams of blood. In lead poisoning, in gout, and in nephritis, the uric acid content of the blood is usually markedly increased and the determination of uric acid in the blood of suspected gout is of unquestioned value.

In connection with gout the recent researches have shown that the old idea that in this condition the blood becomes "saturated" with uric acid must be abandoned. The solubility of uric acid in blood serum has been shown to be much greater than the concentration of uric acid occurring in the blood of gout. Furthermore, in nephritis the uric acid content of the blood may be quite as high as in gout for long periods of time without any symptom of uric acid deposition occurring. We have therefore to assume that in gout there is not only a kidney deficiency for uric acid elimination but that there is also a direct vicarious excretion of uric acid from the blood stream into certain tissues where it finally reaches the saturation point and deposits in the form of sodium acid urate. The view of Minkowski and others that the uric acid circulates in gout in some different form from that in the normal, finds some support from the results already referred to with the blood of lower mammals. It is my opinion that this view will prove to be the correct one.

In connection with the use of salicylates and of atophan in gout it is of interest to note that Fine and others have shown that the administration of either of these drugs to gouty patients is followed by a prompt drop in the uric acid content of the blood. Frequently this drop may be so great that the uric acid practically wholly disappears from the blood for a time. With continued administration of either drug, however, the uric acid reaccumulates in the blood. Hence it is of no service to give salicylates or atophan continuously. Whether by alternating these two drugs for a period of a week or two with each, the blood could be kept relatively free from uric acid continuously, has not yet been determined.

Table No. 7, for which I am indebted to Professor Myers of the Post-Graduate Hospital, illustrates the findings of Myers and Fine in regard to the early accumulation of uric acid in the blood in nephritis. From this table we should infer that of all the common products of metabolism uric acid is the first to accumulate in the blood when the kidney function is impaired. Whether uric acid in high concentration in the blood is *per se* toxic to the kidney is not yet known. The frequency with which gout develops into nephritis might lend support to this view. Occasionally, apparently normal individuals are encoun-

tered whose blood has a uric acid concentration of over 3 milligrams per 100 grams of blood. If such individuals could be followed for some years we would probably obtain data of value as to the possible etiological importance of uric acid in nephritis.

TABLE No. 7.

URIC ACID, UREA N. AND CREATININ OF BLOOD IN GOUT AND EARLY AND LATE NEPHRITIS.

Diagnosis.	Uric Acid.	Urea N.	Creatinin.	Systolic Blood Pressure.
	Mgms. to 100 c.c. blood.			
Typical Cases of Gout.	9.5	13	1.1	230
	8.4	12	2.2	164
	7.2	17	2.4	200
	6.8	14	1.7	
Typical Early Interstitial Nephritis.	9.5	25	2.5	185
	8.0	37	2.7	150
	5.0	37	3.9	130
	7.1	16	2.0	
	6.6	24	3.3	185
	6.3	18	2.1	
	8.7	20	3.6	100
	7.0	33	2.6	117
	6.3	31	2.1	
	6.3	23	2.4	150
Chronic Diffuse and Chronic Interstitial Nephritis.	8.0	80	4.8	240
	4.9	17	2.9	170
	8.3	72	3.2	238
	5.3	21	1.9	145
	9.5	44	3.5	210
	2.5	19	1.9	120
	7.7	67	3.1	
	6.7	17	1.6	165
	8.3	39	2.9	
	6.5	24	3.0	200
Typical Fatal Chronic Interstitial Nephritis.	22.4	236	16.7	210
	15.0	240	20.5	225
	14.3	263	22.2	220
	13.0	90	11.1	265
	8.7	144	11.0	225

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POISONOUS PROTEINS*

(*Continued from page 861, Vol. I.*)

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Part III.—Protein Fever

It has been known for a long time that the parenteral introduction of proteins in the animal body may be followed by fever. As early as 1883 Roques collected the literature of this subject and reported his own experimental studies. A few years later Gamaleia made a most important contribution to this subject. The title of this paper is significant and reads as follows: "The Destruction of Bacteria in the Febrile Organism." Gamaleia found that fever follows the parenteral introduction of bacterial protein, both pathogenic and nonpathogenic, both living and dead, consequently he concluded that fever is a result not directly of bacterial growth, but of bacterial destruction in the body. Indeed, he observed that attenuated bacteria often induce a higher and more persistent fever than the virulent forms. When a rabbit is inoculated with a virulent anthrax bacillus fever develops but persists only a few hours, and then the temperature falls below the normal and death occurs. On the other hand, when the second vaccine is used on a fresh animal, fever appears and continues for three days. When a highly virulent anthrax bacillus is employed there may be no fever and death follows within six or seven hours. Gamaleia made similar observations in other infections and came to the following conclusion: "Fever is not a result of bacterial growth, but on the contrary is consequent upon a reaction on the part of the body against the bacteria and leads to their destruction." Furthermore he found that nonpathogenic bacteria, living or dead, lead to the development of fever. I think that these experiments, made more than a quarter of a century ago, furnish strong support of my theory that fever is due to the parenteral destruction of proteins. One year later this work was confirmed by Charrin and Ruffer and was shown to hold good for nonbacterial proteins as well. In 1890 Buchner induced the characteristic phenomena of inflammation—calor, rubor, tumor, and dolor—by the subcutaneous injection of

*The Herter Lectures for 1916 given in the University and Bellevue Medical School, New York.

diverse bacterial proteins. Krehl and Matthes induced fever by the parenteral administration of albumoses and peptones, but did not obtain constant and uniform results, because as we now know they did not recognize the necessity of regulating the size and frequency of the doses. In 1909 my students and I showed that by regulating the amount and frequency of the dosage we could induce any desired form of fever, acute, fatal, intermittent, remittent or continued.

Inasmuch as I have given elsewhere* the details of this work I will only reproduce the conclusions and make a few general remarks: (1) Large doses of unbroken protein administered intra-abdominally, subcutaneously or intravenously have no effect on temperature, at least do not cause fever. (2) Small doses, especially when repeated, cause fever, the forms of which may be varied at will by changing the size and frequency of the dosage. (3) The effect of protein injections on the temperature is more prompt and marked in sensitized than in fresh animals. (4) The intravenous injection of laked blood corpuscles from either man or the rabbit causes in the latter even in small quantity, either in single or repeated doses, prompt and marked elevation of temperature. (5) Laked corpuscles after removal of the stroma by filtration have a like effect. (6) Protein fever can be continued for weeks by repeated injections, giving a curve which cannot be distinguished from that of typhoid fever. (7) Protein fever is accompanied by increased nitrogen elimination and gradual wasting. (8) Protein fever includes most instances of clinical fever. (9) Animals killed by experimentally induced fever may die at the height of the fever, but as a rule the temperature falls rapidly before death. (10) Fever induced by repeated injections of bacterial proteins and ending in recovery may be followed by immunity. (11) The serum of animals in which protein fever has been induced digests the homologous protein in vitro. In view of recent work on antiferment in blood serum this point needs reinvestigation. (12) Fever is one of the results of the parenteral digestion of proteins. (13) There are two kinds of parenteral proteolytic enzymes, one specific and the other nonspecific. (14) The production or activation of the nonspecific ferment is easily and quickly stimulated. (15) The development of the specific ferment requires a longer time. (16) Sensitization and lytic immunity are different manifestations of the same process. (17) Foreign proteins, living or dead, formed or in solution, when introduced into the blood soon diffuse through the tissues and sensitize the cells. Different proteins have predilection places in which they are deposited and where they are, in large part at least, digested, thus giving rise to the characteristic symptoms and lesions of the different diseases. (18) The subnormal temperature which may occur in the course of a fever or at its termination is due to the rapid liberation of the protein poison, which in small doses causes an elevation, and in larger doses a depression of temperature. (19) Fever per se must be regarded as a beneficent phenomenon inasmuch as it results from a process inaugurated by the body cells for the purpose of ridding the body of foreign substances. (20) The evident sources of excessive heat production in fever are the following: (a) That arising from the unusual activity of the cells supplying the enzyme; (b) That arising from the cleavage of the foreign

*Protein Split Products in Relation to Immunity and Disease, Lea & Febiger, 1913.

protein; (c) That arising from the destructive reaction between the split products from the foreign protein and the proteins of the body.

The above are the conclusions which I drew three years ago from experiments which my students and I had carried out and from a study of the literature of the subject. I did not suppose at the time, nor do I hold now, that all these conclusions are exactly right.

The fundamental fact that the parenteral introduction of proteins may induce fever is founded upon so many independent observations, some of them recorded many years ago, that I do not think it incumbent upon me to seek additional support. Friedberger has, in a most exact way, confirmed the statement that large doses of foreign protein do not, while small doses do elevate the temperature. Moreover, he has shown that a small dose is more effective in sensitized than in unsensitized animals.

Thiele and Embleton have confirmed experimentally the proposition that the parenteral introduction of foreign proteins affects the temperature, causing a rise or fall or having no effect according to the size of the dose. They give the following tables:

EGG-WHITE.

Limits of	NORMAL ANIMAL. grams	SENSITIZED grams
Temperature fall	0.05	0.005
Constant temperature	0.02	0.0002 to 0.0001
Temperature rise	0.01 to 0.001	0.0001 to 0.000002

TUBERCLE EMULSION.

Limits of	NORMAL ANIMAL. grams	SENSITIZED grams
Temperature fall	0.005 to 0.002	0.0005
Constant temperature	0.002 to 0.001	0.0001
Temperature rise	0.001 to 0.00001	0.00001 to 0.000001

Criticism of the statement that foreign proteins find certain predilection tissues in which they accumulate has been made. Iodine accumulates in the thyroid gland. Mercury induces characteristic lesions in the kidneys. Strychnia selects a definite portion of the nervous tissue on which its action is made manifest. The therapeutic effects of the most approved drugs depends upon their predilection for certain tissues. The recent studies of Rosenow indicate that bacterial proteins do not differ from other poisons in this respect. We are accustomed to think of chemotaxis as acting only between morphologically recognizable bodies, but in reality it is a form of chemism and is dependent upon chemical composition and not on histological structure.

The only one of the above given statements formulated some years ago which has met with any experimental negation is my contention that specific proteases are developed by the parenteral introduction of foreign proteins. I am ready to admit that Friedberger's anaphylatoxin comes from the serum. In fact at the same time that I formulated the proposition concerning protein fever I wrote as follows: "It has been suggested: (a) That the agar or kaolin or bacteria absorb the complement from the serum and that this renders it poisonous. (b) That the poison is preformed in the serum, but that its action is neutralized by some other constituent of the serum which is absorbed by the agar

or kaolin. (c) That the absorption of some constituent of the serum by the agar, kaolin or bacteria leads to a disturbance of the equilibrium of the protein constituents of the serum which as a consequence break up with the liberation of the poison. These suggestions assume that the poison comes from the serum and this may be true." On another page I said: "That the anaphylatoxin comes from the blood serum, the one constant factor in all the experiments in its production, is most probable." Now since the probability has become a certainty, we need not conclude that specific proteases never result from the parenteral introduction of proteins. I have shown that all proteins, including those of blood serum, contain a poison and I am not at all surprised on learning that such a poison in the serum is set free in the production of Friedberger's anaphylatoxin and in the development of Abderhalden's pregnancy test, but these have nothing to do with the development of proteases in smallpox or typhoid fever. At least no such connection has been shown.

THE PHENOMENA OF INFECTION.

I have elsewhere gone into some detail concerning the views of the nature of infection which I have developed in my studies on the chemistry and toxicology of bacterial and other proteins. Only a living thing can infect. Injection of diphtheria or tetanus toxin may cause all the symptoms and lesions of the respective diseases, but such injections are artificial procedures and the results are intoxications rather than infections. In this paper I shall omit diseases due to toxins. The infecting agent is a virus and in infections there is a contest between the invader and the native. It is a struggle for food, growth, and reproduction. In the bacterial diseases the structure or the equipment of the invader is quite as complicated and as complete as that of the defender. The contest is between bacterial and body cells and the battlefield may involve only a small part or may extend to every part of the animal's body.

What is the difference between pathogenic and nonpathogenic bacteria? In order for a given bacterium to be pathogenic to a given animal it must be possible for the former to feed upon the latter. All living things feed by means of digestive ferments. Continued life and multiplication are impossible under other conditions. First, in order for a given bacterial to infect a given animal the ferments of the former must be able to digest the proteins of the animal. In the second place the invading cells must not be immediately destroyed by the ferments elaborated by the body cells. There must be a supporting relation between the bacterial cell and the medium, and in infection the body constitutes the medium in which the bacteria grow and multiply. The protein groups split from the medium must fit into the molecular structure of the bacterial cell; otherwise they would be of no service to it. Many kinds of cells may live in the same medium, but for each kind the cleavage of the medium must be specific. From this it follows that the agent by which the cleavage products are secured must be supplied by the cell and must be specific to it.

It follows from what has been said that a bacterium placed in a medium in which its ferment is ineffective cannot grow and multiply. A bacterium which cannot grow and multiply in the animal body cannot cause an infection. Its inability to grow and multiply in the animal body may be due to the fact that its ferments cannot digest or properly break up the proteins of the animal body.

This is one of the reasons why the great majority of bacteria are harmless or nonpathogenic. This, however, is not the sole, and probably not the dominant cause of the failure of so many species of bacteria to do harm to the higher animals. What has been said about the production and utilization of ferments by the bacterial cell is equally true of the body cell. In fact, it is true of every living cell. The body cell has its specific ferments, and the bacterial cell being protein substance is liable to be digested by the ferments elaborated by the body cells. In these simple facts lies the fundamental explanation of all forms of bacterial immunity, either natural or acquired. It will be understood that I am here omitting all reference to the elaboration of toxins and antitoxins.

Ferments are intra- and extra-cellular. All are formed within the cell, but some diffuse into the medium while others do not. In some instances at least, cell permeation by the pabulum is essential to the feeding of the cell. In other cases the ferment accumulates on the surface where digestion proceeds. In others the ferment diffuses into the medium more or less widely from the cell which produces it. Many cells produce both intra- and extra-cellular ferments, and these differ in function.

I am not going into detail concerning cellular ferments. Those of the bacterial cells are easily obtained and have been studied quite elaborately. Some digest proteins, such as gelatine, quickly while others are less prompt and others still have no recognizable effect on this protein. They are easily affected by the presence of certain nonprotein substances, especially carbohydrates. The ferments of the body cells are not so easily obtained and are more difficult of study. However, both the intra- and extra-cellular ferments of the polymorphonuclear corpuscles have been studied in some detail and their destructive action on certain bacteria has been demonstrated. The germicidal action of the blood and its serum has been demonstrated on various species of bacteria.

It may be well to point out some differences between intra- and extra-cellular ferments. The latter are comparable to the enzymes of the alimentary canal. Their function is solely a lytic one. They break up complex proteins into simpler bodies, but these without further treatment are not ready to be built into the cellular structure. The extra-cellular ferments are in a general way destructive in action. The intra-cellular ferments are essentially constructive. They shape the rough blocks and fit them into the molecular structure. In the process of infection the intra-cellular ferments of the bacterial cells are most active. The soluble, simple proteins of the fluids of the animal's body are quickly built into the bacterial cell and growth and multiplication result. Body proteins are converted into bacterial proteins. This process proceeds so smoothly that as a rule during the time when its development is most rapid the host is quite unaware of the presence of his undesired guest. Whole molecules of albumins and globulins are taken into the bacteria and built into the more complicated bacterial cell. This is the period of incubation in an infection. The body cells are not prepared to combat the invader during this period. Finally the body cells react and begin the elaboration of ferments which destroy the bacterial proteins. This is quite a different process. Complex, cellular proteins are split into simpler ones and protein poisons are set free.

During the period of incubation of an infectious disease, the infecting organism supplies the ferment, the simple, soluble proteins of the body fluids con-

stitute the substrate, the process is essentially constructive, no poison is set free and there are no recognizable clinical symptoms. During the active progress of an infectious disease, the body cells supply the ferment, the complex, bacterial, cellular proteins constitute the substrate, the process is essentially destructive, the protein poison is set free, the symptoms of disease appear, lesions more or less destructive develop and life is placed in jeopardy.

The experienced clinician will easily understand that in most infectious diseases the steps in the evolution of the processes are not so clearly defined as indicated in the above statements. They are most typical in uncomplicated cases of yellow fever, typhoid and typhus and in smallpox, but even in these there often are complicating factors. In yellow fever an attempt is made to eliminate the poison into the alimentary canal as is evidenced by black vomit. In typhoid the poison in being excreted into the intestine may lead to perforation. In most infections, the bacterial growth and their disruption overlap. In one part of the body the bacteria continue to grow while in other parts they are being destroyed. In pneumonia life may be endangered by the abundance and extent of the exudate, while in the crisis of this disease autolysis probably plays an important role not only in the destruction of the organisms, but in the removal of the exudate. In many infections lesions develop and impair the efficiency of the body cells. Moreover in destructive lesions the dead tissues of the body must be disposed of and this throws an increased burden on the body cells. In some diseases phagocytosis plays an important role. It must be evident that the engulfment of bacteria by phagocytes is a more conservative method of disposing of the invading cells than their extra-cellular destruction, since in the former the body is protected against the poison liberated by bacterial cleavage. Nothing more dangerous to the infected individual could happen than the sudden cleavage of all the bacteria in his body. The poison liberated in this process would overwhelm him at once. This is a probable explanation of the fact, already referred to, that the case mortality in typhus fever is higher among the well nourished than among the less robust. Bacterial cells, as well as body cells, have means of protecting themselves. The tubercle bacillus through limitless generations of parasitism has developed coatings of fats and waxes which protect them against the action of secretions of body cells quite as efficiently as coats of mail protected our ancestors against the weapons of their time. Moreover, bacterial cells may develop increased resistance or become to some extent immune to the action of body cell secretions. Occasionally bacteria persist in the body for long periods after recovery from the disease and when these are transferred to new hosts they show that they have lost nothing in virulence. Frequently, secondary infections develop and decide the fate of the individual. As someone has said the pyogenic micro-organisms frequently play the last act in the great tragedies of life, tuberculosis, cancer, and syphilis.

A CHEMICO-BIOLOGIC CONCEPT OF THE PROTEIN MOLECULE.

Under this heading I wish to formulate certain theories which have developed in my mind during the progress of the work which I have outlined in preceding lectures. Some men seem able to work without developing theories and probably this is best, but I have never worked in that way. It is possibly a fault; if it be, I am ready to confess that I have sinned and continue in the

same old way. I hope that some of the statements which I am about to make will stimulate others to investigate and this I deem of more importance than their truth or falsity.

The protein poison about which all my work has centered is a fact. It has been prepared and studied by so many competent men that its wide distribution in proteins from diverse sources cannot be questioned. Its effects on animals have been widely tested and the general conclusions reached are quite as uniform as those which might be formulated about poisons much longer known. Its chemical structure has not been determined with certainty. The best evidence at hand today seems to indicate that it is not a basic body, and therefore not a protein alkaloid, not a leucomain or a ptomain. It contains no phosphorus and no carbohydrate. In the purest form in which it has been obtained it yields a trace of ash of which phosphorus and chlorine are not essential constituents. Whether this mineral matter is an essential part of the poison or not I do not know. Under any condition in which it has been obtained it is decidedly acid in character and yields amino acids on disruption. It seems to be a polypeptid.

Underhill, whose opinion I esteem highly, concludes that the action of the protein poison on animals is similar in kind but more intense than that of proteoses. I dare say that this is quite right and it conforms with my own observations. I suggested in the Shattuch lectures in 1906 that the protein poison is the chemical nucleus, keystone, or archon of larger and more complicated protein molecules.

The chemism of the protein poison is intense and it combines with various inorganic and organic substances to form more complex molecules, still retaining and imparting to these larger molecules its protein characteristics. Combined with phosphate of lime it forms such phosphoglobulins, so called, as casein. Combined with carbohydrate it develops the glycoproteins and in combination with both phosphorus and carbohydrates, the glyco-nucleo-proteins result. In the last mentioned bodies the protein molecule reaches its most complex form and further development is possible only by polymerization and the aggregation of many protein molecules into cells. At what stage in the evolution of the protein molecule metabolism begins I cannot say, but it is quite evident that multiplication does not begin until the most complex structure has been reached. It seems quite evident that from the beginning the process is a synthetical one.

It is possible to conceive of the beginning of life on the earth, as proceeding in this way. In the intense heat of past geological ages when even carbon existed in the gaseous state this element combined with nitrogen forming cyanogen. With this binary compound under proper conditions the synthesis of the simplest amino acid was possible for cyanogen may react with boiling hydroiodic acid with the development of amino acetic acid and from this the other amino acids found in the protein molecule might have been developed. In this view, proteins in their simplest form may have come into existence long before life as we now know it was possible on the earth.

The simplest protein, as the protein poison, has its intense chemism satisfied as it combines with other elemental groups in the development of the more complex bodies.

I began my work with the hope of finding simple proteins in the cellular structures of bacteria. In this I was disappointed and I now see that I should not have expected it. Instead of finding simple proteins in bacterial cells I have found them in the casein of milk and in the proteoses of seeds. As I have already said the young mammalian is fed upon food principles served in the simplest form. The nursing child is supplied with fats as such, with mineral constituents for the most part uncombined, with carbohydrates in the easily assimilable form of lactose and with amino acids in the relatively simple protein, casein. The sprouting seed finds the amino acids with which it starts life in the relatively simple proteins while fats and carbohydrates are supplied in a ready-made form. Now if this provision be made for the support of the developing plant and animal, what can be said about the food supplied the numerous cells of the body, whether it be plant or animal. Simple proteins exist in the circulating blood of the higher animals. Not only is this true but as Van Slyke and his coworkers have shown the body cells directly use amino acids. The simple proteins probably exist in the circulating blood chiefly in that protein mixture about which we know but little and which we designate as serum globulin. In this mixture the primitive proteins are ready to enter into combination with the more complex cellular proteins as the latter wear away in their functional activities. Their chemism is held in abeyance by combination with some indifferent substances, such as calcium. I have found that the protein poison from casein is neutralized in vitro by calcium lactate. Indeed the protein poison is largely, but not so quickly, neutralized by incubation with sodium bicarbonate. In this connection it may be well to recall the effect of the withdrawal of calcium on the coagulation of blood and that after severe poisoning with the protein body the clotting of the blood is retarded and often wholly prevented. If my idea that the circulating blood at all times contains the protein poison from the too violent chemism of which the body is normally protected by its combination with an inert body, it will not be difficult to understand that the equilibrium may be disturbed in a variety of ways with death as a result. The introduction of a little more of the poison or the removal of the protecting body may seriously upset the equilibrium. Casein yields about ninety per cent of its weight in protein poison. The calcium is easily removed from casein. An ash-free casein may be prepared by repeated solution in dilute ammonia and reprecipitation with dilute acid. The last trace of calcium is removed by treatment with oxalic acid. The protein poison from casein resembles the globulins inasmuch as it may be wholly precipitated from aqueous solution by saturation with sodium chloride, but differs from globulins inasmuch as it is freely soluble in absolute alcohol.

Blood is rendered poisonous not only by incubation with bacteria, agar, starch, kaolin, etc., but as was shown by Köhler as long ago as 1877, it becomes poisonous on clotting, killing both homologous and heterologous animals. This phenomenon which has been confirmed by others has recently been investigated by Moldovan who has shown that blood freshly defibrinated by shaking with glass beads causes acute death when injected intravenously into guinea pigs and rabbits. In the former the typical anaphylactic lung picture is seen after death. When the dose is slightly sublethal there is marked fall in temperature with subsequent fever. When the doses are smaller there is marked fever. On stand-

ing from fifteen to forty-five minutes defibrinated blood loses its toxicity. Serum obtained by rapid centrifugation of defibrinated blood is poisonous. The same is true of the deposited and once washed corpuscles. When coagulation is delayed by the presence of sodium citrate neither the supernatant fluid nor the corpuscles are poisonous, but both become so when coagulation is induced by shaking with porcelain beads. Doerr has shown that blood received in paraffined vessels becomes poisonous; but when the coagulation is complete the toxicity disappears. When coagulation is made to proceed slowly by the addition of hirudin solution or a 0.7 per cent solution of colloidal silicic acid, it retains its toxicity for several hours.

The fact that extracts of normal tissue, when injected intravenously, are poisonous is another interesting fact. If the lungs of a rabbit be macerated for two hours in salt solution, the solution kills promptly on intravenous injection. Homologous organ extracts are more poisonous than heterologous.

All these phenomena show that there is under normal conditions a nice adjustment in the constituents of blood and tissue whereby life is protected and that slight changes easily disturb this equilibrium with most disastrous results. There are here unsolved problems but my work leads me to the conclusions that there are protein bodies in the blood and tissue, which serve under normal conditions as cell foods, but which may become explosively poisonous when the mechanism regulating their use is disturbed. Normal cells contain deposits of these bodies, which under proper regulation, supply cell waste, but under abnormal conditions lead to cell destruction. These substances were probably present in my bacterial cells, but I washed them out and threw them away leaving only the cellular proteins. However, time and labor will solve these problems and I turn to another phase of my subject.

If I properly interpret my work on the chemistry of bacterial proteins it confirms the theoretical views of Pflüger, Ehrlich, and Verworn, who have held that the essential part of cells consists of a chemical unity, made up of giant molecules. So far as I can find this view receives additional support in the experimental work done by others. I have been able to find but little upon this subject. Reinke and Rodewald found that air dried substance of æthylium septicum, which they designate as plasmodium, consists largely of highly complex proteins containing phosphorus and yielding xanthine bases and carbohydrates on disruption. Sosnowski concludes from his study of infusorial cellular substance that this does not contain simple proteins as such, but as constituents of highly complex molecules. My studies have led me to formulate a theory concerning the nature and operation of living matter. My first attempt in this direction was made in a lecture delivered in Toronto (1905), and this was elaborated in a Shattuck lecture (1906). The cell is not the unit of life; life is molecular. Life is function, not form. The cell is not only made up of protein molecules, but its form and function are determined by the chemical structure of its constituent molecules. The lines along which the spore, seed or ovum develop are determined by the chemical structure of its proteins. Growth in other directions is impossible, and this accounts for stability in reproduction. However, changes in the chemical structure may and do occur and in these lies the basis of variation.

The keystone or archon of the protein molecule is the protein poison. It is common to all protein molecules. Physiologically it is the same in all molecules, i. e., when set free it is a poison and it is a poison on account of its intense chemism which enables it to tear off groups from other proteins. One protein differs from another in its secondary and tertiary groups. Most native proteins are not poisonous because in them the chemism of the primary group is satisfied by combination with secondary groups. Strip off the secondary groups and the primary becomes poisonous on account of the avidity with which they combine with the secondary groups of other molecules. Biological relationship between proteins depends upon the secondary groups. In this way varieties and species have developed.

The living molecule is never in a state of equilibrium. There is a constant exchange of atoms between it and the outside world. It absorbs, assimilates and eliminates. It is constantly trading in energy. It takes in oxygen and gives off carbonic acid; it takes in nitrogenous material and having utilized it the waste is discarded. The living molecule passes through the period of growth and decay. During the former its functions are largely synthetic; in the latter they are autolytic and finally the structure drops into pieces.

(Concluded.)

A STUDY OF THE TESTS OF LIVER FUNCTION*

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IN order to study the functional activity of an organ, it has been customary to apply certain specific tests to the individual functions of that organ. Thus if an organ has several functions, tests are applied to one of these functions, and conclusions are drawn therefrom as to the capability of the organ to perform all of its offices. This has been especially the case in the investigation of the condition of the liver.

The liver has a multiplicity of duties to perform in the body, all of which are of essential importance. It is possible that each individual cell of the hepatic structure takes part in all of the liver functions; it is also possible that different portions of the liver lobule, and different conglomerations of the liver lobules may have specific functions. In the former case, it is most likely that a reduction in the ability of the liver to perform one function will be accompanied by a proportional reduction in all the liver functions; in the latter case, one or more functions of the liver may be disturbed without affecting the other hepatic functions.

In order to appreciate the various methods for determining the liver functions, it is best to enumerate the different functions of the liver:

1. Secretion of bile.
2. Relation to carbohydrate metabolism.
   a. Glycogen formation.
3. Relation to nitrogen metabolism.
   a. Formation of urea.
4. Detoxification function.
   a. Formation of the conjugate sulfates and glycuronates.
   b. Withholding of toxins and poisons.
5. The decomposition of the erythrocytes.
6. The formation of fibrinogen.
7. The formation of antithrombin.

The methods for the study of the liver functions are several. These tests can be classified in the following way:

1. A study of the carbohydrate tolerance of the liver; this will include the tests of general carbohydrate metabolism; tests of tolerance for special carbohydrates, for example, Bauer's galactose test, Strauss's levulose test, etc.

2. A study of the nitrogen excretion in the urine, including the urea, amino, and ammonia nitrogen fractions.

3. The urobilinogen excretion in the urine, which von Jaksch, in 1892, considered significant of liver disease.

4. Analysis of the fibrinogen of the blood, which was found to disappear

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from the blood after liver extirpation (Doyon and Kareff, Nolf, Corin and Ansiaux, etc.).

5. A study of lipase and fibrinolytic ferments of the blood (Whipple, Mason and Peightal, Goodpasture).

6. The phenol tetrachlorphthalein test (Rowntree, Hurwitz and Bloomfield; Kahn and Johnston; McLester and Frazier).

1. *Carbohydrate tests of hepatic function.*

a. Bauer tested the liver function by administering to the patient 30 grams of galactose. The urine is then analyzed for galactose. If present the liver is not functionating properly.

b. Strauss used another carbohydrate—levulose—for this test. He administered 100 gms. of this substance per os and then analyzed the urine for levulose by Salmanoff's reaction.

2. *Study of the nitrogen metabolism.*

It is well known that the liver plays an important role in the protein metabolism of the animal organism. Disturbances of liver function will induce deviation from the normal of the nitrogen metabolism. Rowntree, Marshall and Chesney have found a low percentage of urea and a high percentage of amino acids and ammonia in the urine of patients suffering from hepatic involvement. It is known that in eclampsia, with liver necrosis, there is always a high ammonia coefficient.

3. *Study of the urobilinogen excretion.*

Von Jaksch thought that the presence of urobilinogen in the urine was indicative of hepatic disease. The studies of Wilbur and Addis have definitely demonstrated that such is not the case. The urobilinogen is tested for by Ehrlich's para-dimethyl-amino-benzaldehyde.

4. *The determination of the fibrinogen content of the blood* (Whipple) and of the fibrino ferment of the blood (Goodpasture) were found to be valuable by Rowntree, Marshall and Chesney.

5. *The determination of the lipase of the blood,* as recommended by Loevenhart, has not proved of any distinct value.

6. *The phenoltetrachlorphthalein test.*

In 1909, Abel and Rowntree conducted pharmacological experiments on animals with phenoltetrachlorphthalein, which was synthetized by Professor Orndorff, of Cornell University. They found that this substance, when injected intravenously, was excreted in the bile. At the suggestion of Rowntree, Whipple, Mason, and Peightal studied the excretion of this substance in the bile when the liver was subjected to artificial lesions. These authors found that in dogs which had been poisoned by phosphorus, for example, the excretion of the phthalein was interfered with. It was then that Rowntree, Marshall and Chesney applied the tests clinically and obtained rather encouraging results.

The phenoltetrachlorphthalein test is applied in the following manner:

The dye is to be prepared for use each time. One gram of the substance is placed in a 200 c.c. Erlenmeyer flask, with 2 c.c. of 2/N sodium hydroxide solution and 18 c.c. of freshly distilled water. This is boiled for twenty minutes under a reflux condenser. The solution is filtered into a 100 c.c. flask, and is ready for use. This gives approximately a five per cent solution, which is al-

most isotonic with blood. The solution is of an intense purplish color; it will not keep for more than a few days. Arbitrarily 8 c.c. of this solution, approximately 400 mgm., of the phthalein has been selected. This amount is sufficient to give a most intense purplish-red color to twenty liters of water. Its administration in health is never followed by the appearance of the dye in urine, and this amount insures in health an intense color in the final preparation of the feces, which is used for the quantitative determination. The dye is administered intravenously by gravity with antiseptic and aseptic precautions and with the usual intravenous technic. The funnel and system are filled with freshly distilled water, and after the flow is well established the phthalein solution is added. Fifty to 100 c.c. of water are used and the phthalein solution is washed in with freshly distilled water until the fluid entering the veins is colorless. Ten to fifteen minutes are required for its administration. Physiological salt solution may be preferable to distilled water for use in this injection.

Active purgation is instituted prior to the administration of the dye, and throughout the time of observation, usually by means of compound cathartic pills. The stools are collected for forty-eight hours, the urine for twenty-four hours. In the event of little or no feces being obtained, enemata are used, but unless a normal amount of dye is recovered the test must be discarded, since low findings under this condition could not be accepted.

The total forty-eight hour feces are placed in a two liter bottle and diluted with water to one or 1.5 liter, depending on their amount. This is placed in a shaking machine for from five to twenty minutes. Without allowing time for sedimentation, one-tenth of the total is placed in a one liter flask and to this is added 5 c.c. of forty per cent sodium hydroxide, which causes the mixture to take on a very red color. Dilution is made with water to one liter. A stopper is inserted and the mixture thoroughly shaken. One hundred c.c. of this preparation is placed in a 200 c.c. flask, 5 c.c. of lead acetate added, resulting in a discoloration of the mixture and a throwing out a heavy lead precipitate which carries down all the pigments, leaving a clear colorless supernatant fluid. Five c.c. of forty per cent sodium hydroxide are added; this again elicits the red phthalein color, but does not redissolve the other lead pigment combination. In certain instances 5 c.c. of sodium hydroxide at this point are not sufficient to elicit the maximum intensity of red, and more should be added until maximum intensity is reached, but not sufficient to free the other pigments from their insoluble lead combinations. The contents of the flask are made up to 200 c.c., shaken, and, a small part filtered off, or the solution is allowed to stand for five minutes, when in many cases a clear red, supernatant fluid ready for estimation can be decanted. This solution is compared in a Rowntree and Geraghty modification of the Autenreith and Koningsberger colorimeter with 20 mgm. to a liter solution of the disodium salt of tetrachlorphenolphthalein (e. g., O.4 c.c. of original solution to one liter, plus sufficient sodium hydroxide to insure maximum color). With these dilutions the amount of dye present is indicated directly in percentages.

When the amount recovered is below normal, it is advisable to add 2 to 3 c.c. more alkali to the 200 c.c. preparation, and redetermine, thus insuring that the maximum color has been elicited. The addition of large quantities of akla-

lies is undesirable, since it sets free the other pigments, rendering the solution yellowish-red instead of purplish-red. Not more than ten minutes are required to carry out this test after the feces are removed from the shaker. Where difficulty is experienced on account of the quality of the color, the following procedure may prove of some value in certain instances: After the addition of about 10 c.c. of forty per cent sodium hydroxide, the feces are made up with water to one liter. To one-tenth of this is added five c.c. sodium hydroxide and water up to one liter. Of this 100 c.c. are placed in a 200 c.c. flask and to it are added 5 to 10 c.c. or more of calcium chloride mixture until the best quality of color is elicited. Dilution is made to 200 c.c., the mixture is allowed to stand from one-half to twenty-four hours, and a small amount of the supernatant fluid is filtered off and read against the standard.

7. *A study of sulfo-conjugation as influenced by liver diseases.*

The cause and the location of the formation of the ethereal sulfates and of indican has been studied by a number of investigators.

Since Städeler found phenol in cow's and horse's urine, Landolt, Lieben, Hoppe-Seyler, Buliginsky and Munk found traces of it in normal human urine, and Salkowski observed that in ileus and other obstructive intestinal disease, the excretion of phenol in the urine is much increased.

This formation of phenol and phenolic substances, cresol, indol, skatol, etc., has been ascribed to the action of the intestinal bacterial flora. Such organisms like the B. coli communis, which is a normal inhabitant of the intestinal canal, are harmless under normal circumstances. In conditions of injury to the intestinal mucosa, these organisms become virulent (Fermi and Salto). Other organisms, like the B. Putrificus, B. ærogenes capsulatus, which are obligatory anaerobes thrive in the colon where there is no oxygen (Herter), and break up protein into the carbocyclic, toxic substances.

It was demonstrated by Baumann that these split products are very toxic, but that when they are united with sulfuric acid, they have lost their poisonous effect.

Baumann found that phenol sulfate is a normal urinary constituent and that the administration of phenol increases the phenol sulfate in the urine.

Baumann and Herter reported that not only phenol, but also other substances were excreted in the urine as ethereal sulfates. They also observed that phenol unites not only with sulfuric acid but with other radicals. This was confirmed by Schmiedeberg, who found that phenol unites with glycuronic acid.

Upon poisoning dogs with phenol, he found that the liver became rich in phenol sulfates. For example in 100 parts of liver he found 19 times as much tribrom phenol as in 100 parts of blood. This phenomenon seemed to prove that the liver is the seat of conjugation of the phenolic and indolic radicals with sulfuric acid.

Lang determined the quantity of ethereal sulfates in the urine of geese before and after extirpation of the liver. His figures are rather small, and should not be taken conclusively, but he was led to believe that the synthesis of the ethereal sulfates was not exclusively performed in the liver.

In experiments, performed in vitro, Kochs also demonstrated, so it appeared to him, that the liver was not the only seat of sulfo-conjugation. He took

liver, kidney, pancreas, thymus, muscle, and minced each organ respectively, and added phenol and disodium sulfate. He kept these mixtures at body temperature or else at 8° to 12° C. He reported that all the tissues, save the thymus, took part in the synthesis. He obtained similar results with ortho-, meta-, and para-di-oxy-phenol.

Landi repeated the experiments of Kochs, using only the liver tissue. But, as he says, due to the fact that decomposition sets in so very soon, he could not confirm Kochs' findings. In order to throw more light on the subject, he made perfusion experiments with the liver, and he came to the final conclusion that the seat of conjugation of the phenolic and sulfuric radicals was not the liver but the intestines.

The results of Landi are directly negated by the findings of Embden and Glaessner. They performed perfusion experiments on the organs of dogs, using the liver, muscle, kidneys, lungs and small intestine. From their investigations they conclude that the liver is the most important organ for the formation of the ethereal sulfates. Smaller quantities of ethereal sulfates are produced in the lungs and the kidneys, but the muscle tissue and the small intestine play a very insignificant role in the formation of the ethereal sulfates.

Reale, from his observations, was of the firm opinion that the liver was the seat of the synthesis of the ethereal sulfates.

Finizio confirmed Reale from his clinical findings. In normal individuals and in a case of echinococcus hepatic cyst, he found that the administration of thymol caused an increased excretion of ethereal sulfates in the urine. When, however, he administered thymol to a patient suffering from hepatic cirrhosis, he found no increase of the ethereal sulfates in the urine.

In normal conditions of the alimentary tract, Strauss and Philipsohn found no phenol in the urine, and they concluded that under normal conditions, the phenol and other radicals were conjugated with sulfuric acid. According to these authors, the liver is the seat of the synthesis of the ethereal sulfates.

Herter and Wakeman took 7 gms. of liver, kidney, muscle, brain, and blood respectively, which were minced, and treated each tissue with 10 c.c. of a weak phenol solution, and allowed to stand for two to three hours. The mixtures were then distilled, and they found that there was a loss in the phenol distilled over. The liver retained most of the phenol, then came in order the kidneys, muscle, brain.

In conditions of jaundice, Biernacki found four times as much ethereal sulfates as normally. Darenberg and Perroy found an increased excretion of indol and skatol in jaundiced individuals. Labbe and Vitry obtained similar results. Magrageas obtained varying quantities of ethereal sulfates in icteric patients.

Amann found that in the healthy subject there is a direct proportion between the quantities of ethereal sulfates and the total nitrogen in the urine. The coefficient of Amann may be thus expressed.

$$\frac{\text{Eth. S.} \times 100}{\text{N. Urine}}$$

The value of this coefficient varies between 1.4 and 1.5. This was confirmed by Guerbet and Rouen. Slightly smaller coefficients were obtained by Magrageas.

The question has been discussed by Eiger and Hopadze whether the aromatic compounds formed in the system are diminished in amount and destroyed under normal conditions of hepatic activity, and whether, in cases of disturbance of the function of the liver, these compounds are obviously increased and placed at the disposal of the liver for conjugation with sulfuric acid. The subject is more important in its relation to cases of disease of the hepatic parenchyma than to simply biliary stasis. The ethereal sulfuric acids are most frequently, both absolutely and relatively, increased in atrophic cirrhosis of the liver, and most markedly in tumors of the liver.

In normal urine 14 to 25 per cent of the total sulfur is present as the so-called neutral sulfur. The easily oxidizable portion of this must arise from the sulfocyanate of the saliva, and from other partly unknown substances, while the remainder is regarded—in part, at least—as a derivative of the taurin of the bile (Lepine). This latter bears, in the nomenclature of the French physiologists, the name "biliary sulfur of the urine."

Lepine found, in incipient cases of obstructive jaundice in animals and in man, the biliary sulfur absolutely and relatively increased as regards the oxidized sulfur (up to 30 to 43 per cent of the total sulfur). After a few days of the biliary obstruction, the sulfur became approximately normal, and after long continuance of the disturbance showed a decrease.

Regarding the fate of taurin and the origin of the neutral sulfur in the body, the with difficulty oxidizable neutral sulfur cannot yet be regarded as the amount of formed, absorbed, and decomposed taurocholic acid. For instance, it has been shown that both components of the neutral sulfur vary within the widest limits in spite of feeding with the same amounts of food, and notwithstanding the same external relations of the animals used in the experiments, so that the special relation of the with difficulty oxidizable sulfur to taurin becomes rather doubtful (Benedict). Nevertheless, attention must be called to the fact that the early increase and later decrease of the neutral sulfur described by Lepine is very comparable to the view which we must take regarding the process of the formation of biliary acids in jaundice.

The following example, selected from Lepine's work on cholelithiasis illustrates the course of excretion of neutral sulfur in jaundice:

May 2: Light jaundice.
" 3: Light jaundice; neutral sulfur=31 per cent.
" 6: Sudden increase of jaundice.
" 7: Marked jaundice, neutral sulfur=43 per cent.
" 10: Marked jaundice; neutral sulfur=20 per cent.
Total sulfur=100.

F. Muller, who studied a case of jaundice from gall-stones of somewhat long standing, found on three days the values of the neutral sulfur to be 22.9, 15.7, and 10.7 per cent of the total sulfur. Later in the same case, but with different diet, the values were 19.2 and 17.4 per cent. In a case of carcinoma of the stomach and liver, accompanied by jaundice, the findings were 29.0, 21.1, and 16.1 per cent. These figures confirm Lepine's idea that the neutral sulfur diminishes the longer the jaundice continues.

On the other hand, a marked decrease, and even a lowering of the normal values, should be expected in chronic obstructive jaundice, provided the assumption is correct that in cases of disturbed outflow of bile into the intestine the production of biliary acids is markedly reduced by the interruption of the circulation of bile acids. Since this is not observed, the relation of the hardly oxidizable sulfur to taurocholic acid must be reinvestigated before an opinion on the formation of bile acids can be based on the excretion of neutral sulfur. Hence it does not follow that Schmidt should assume that the production of bile acids, even in long continued jaundice, suffers no reduction, because he but rarely found high values for the neutral sulfur in his case of jaundice. According to Benedict, a portion of the non-oxidized sulfur compounds, which may be excreted in increased amounts as a result of toxic action on the protein constituents of the body, are to be regarded as intermediary bodies, which resist the further oxidation to sulfuric acid. Corresponding to their presence in the bile (Bial), conjugated glycuronic acids are regularly observed in the urine in cases of biliary obstruction (van Leersum) [Von Noorden: Metabolism and Practical Medicine].

We studied the liver function by several of the above methods.

1. *The tetrachlorphenolphthalein test.*

We applied this test in a series of thirty-four cases. This series included patients who were suffering from liver disease, as well as those who had no hepatic ailment. The test is not as easy to carry out as the description indicates. It is rather difficult, and in many case almost impossible to impress the nurse with the importance of collecting the entire quantity of feces. The duty is rather a disagreeable one and complaints are likely to arise. The chemical analysis is also a disagreeable procedure and in a number of instances almost discouraging. In these cases it is almost impossible to obtain a color which can be compared with the standard. In general this test is not easy; it requires some experience, and it needs a well equipped laboratory.

The accompanying table shows the percentages of dye recovered in the various cases. Several cases which clinically were typical cases of liver involvement gave rather high figures for the phthalein excretion in urine, whereas other cases in which the diagnosis pointed to nonhepatic involvement, there was frequently observed a very low phthalein output.

We concluded that this test is of very doubtful value. It certainly does not lend itself to clinical purposes. It is difficult of performance; the manipulations are very disagreeable; and the results obtained not conclusive.

2. *The Urinary nitrogen partition.*

Rowntree, Marshall and Chesney concluded from their experiments that the nitrogen partition is of distinct value in determining whether or not there was a disturbance in liver function. We have obtained similar results. In eclampsia of pregnancy, where the liver is involved we found always an increased ammonia nitrogen, and amino acid nitrogen output, and a decreased urea nitrogen elimination. Normally the ammonia nitrogen is about 4 to 5 per cent of the total urinary nitrogen, and the urea nitrogen is 80 per cent of the total nitrogen. In eclampsia and in severe disease of the liver, the ammonia output may be increased to 40 per cent or more at the expense of the urea nitrogen.

NO.	NAME.	DIAGNOSIS.	OUTPUT OF PHTHALEIN, PER CENT.	REMARKS.
1	B.	Fracture	14	
2	B.	Gastritis	28	
3	B.	Fracture	31	
4	S.	Mitral insufficiency	19	
5	H.	Jaundice	5	
6	B.	Fracture	12	
7	S.	Congestion of liver	32	
8	B.	Congestion of liver	34	
9	D.	Cholelithiasis	28	
10	S.	Chronic gonorrhea	25	
11	H.	Amputation	22	
12	R.	Hernia	27	
13	K.	Renal tuberculosis	32	
14	K.	Burns	24	
15	D.	Tuberculosis	21	
16	S.	Malaria	34	
17	D.	Cholelithiasis	20	Jaundice
18	F.	Cholecystitis	21	
19	R.	Fracture	14	
20	R.	No diagnosis	23	
21	D.	Liver congestion	25	
22	H. V.	Atrophic cirrhosis	25	
23	W. J.	Atrophic cirrhosis	17	
24	E. P.	Atrophic cirrhosis	21	
25	H. C.	Syphilis of liver	24	
26	C. L.	Abscess of liver	19	
27	T. M.	Cholecystitis	25	Jaundice
28	P. A.	Cholecystitis	30	
29	R. S.	Gall-stones	18	Jaundice
30	H. O.	Gall-stones	27	Jaundice
31	W. K.	Gall-stones	26	
32	L. Y.	Cancer of liver	17	
33	H. P.	Chronic gastroenteritis	32	
34	G. L.	Mucocolitis	35	

3. *The lactose and levulose tests* have been of no value to us as an aid in the diagnosis of liver disease.

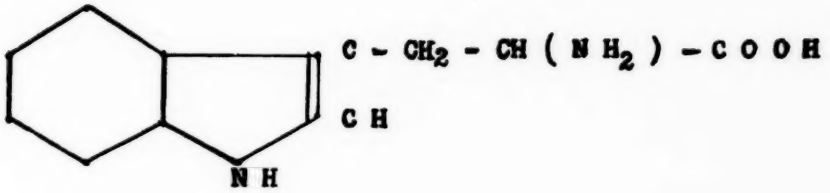
4. *The urobilinogen test* may be classed with the carbohydrate tolerance tests.

5. *The sulfo-conjugation.*

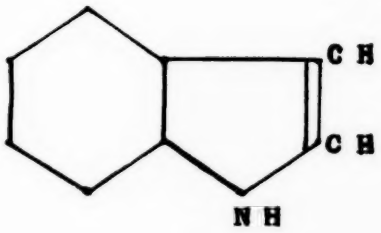
This has helped us much in determination of liver function. We shall discuss this in detail.

The toxic aromatic radicals produced by decomposition of protein are conjugated in the liver with sulfuric or glycuronic acid, and are then excreted in the urine. If we should take indol as an example, the following process would take place.

Tryptophane, or beta-indol-alpha-amino-propronic acid is one of the products of decomposition and putrefaction of proteins. It is the mother substance of indol and skatol, etc. Upon breaking down of tryptophane, indol, which is very toxic is produced.

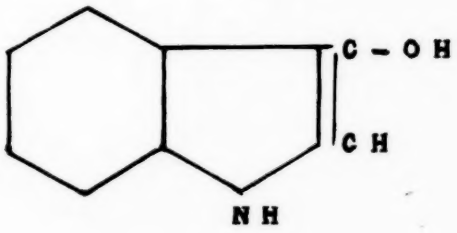


(Tryptophane)



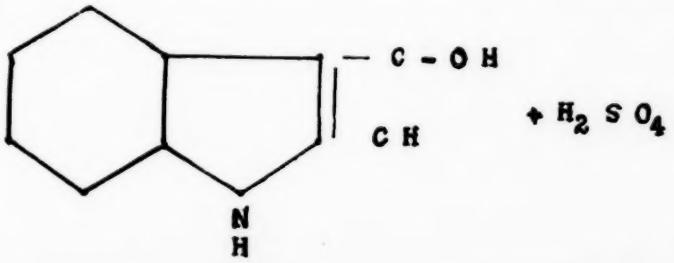
(Indole)

Indol is oxidized in the intestines to indoxyl.

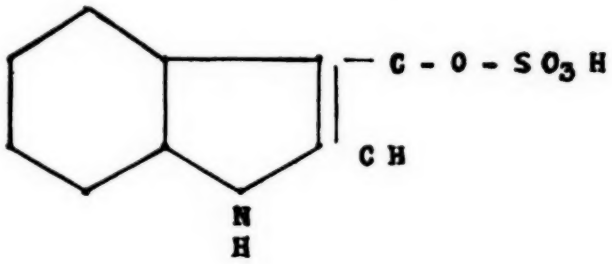


(Indoxyl)

If indol or indoxyl enters the general circulation marked toxinemia results with its concommitant symptoms. The protective mechanism of the body against this toxinemia is to conjugate the indoxyl with sulfuric acid in the liver, producing a substance which is almost nontoxic—indican.

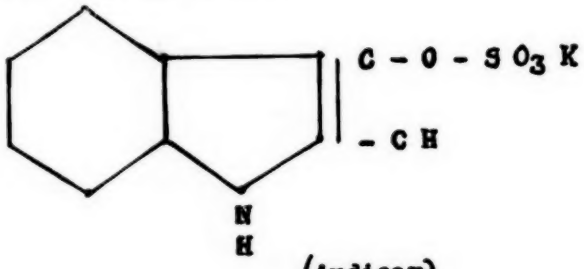


(indoxyl)



(indoxyl sulfuric acid)

In the presence of potassium salts:



(indican)

Similar results are obtained with any of the aromatic radicals, as phenol, cresol, tryosin, skatol, etc.

It is well known that the total sulfur in the urine may be partitioned into three distinct fractions:

a. The Inorganic Sulfates.
b. The Ethereal Sulfates.
c. The Neutral Sulfur.

It has been definitely established that, normally, the inorganic sulfates form about 70 per cent of the total sulfur, and the remaining 30 per cent are divided almost equally between the ethereal sulfates and the neutral sulfur.

The ethereal sulfates are the conjugated aromatic sulfonic acids. It is this fraction that is of special interest to us now.

It is, of course, impossible to rely upon the excretion of ethereal sulfates as a symptom of hepatic function. The proteins which are ingested daily give rise to their quota of aromatic radicals which influence the quantity of the conjugated sulfates. The condition of the intestinal flora plays a role in the formation of aromatic radicals, thus it is known that in intestinal putrefaction there is a marked increase in the conjugated sulfates excreted.

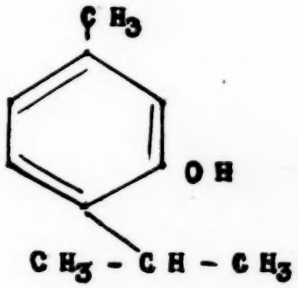
We, therefore, adopted the following technic for the determination of liver function by means of the ethereal sulfate output:

The patient received a dose of castor oil to clean out his bowels. He was then kept on a known diet for two days, during which time the urine was collected, preserved, and analyzed for total sulfur and ethereal sulfates*. On the

*The total sulfur was analyzed by Benedict's method; the Ethereal sulfates by Folin's method.

third day the patient received a capsule containing one-half gram of thymol. The urine was collected for the next two days, preserved, and analyzed for total sulfur and ethereal sulfates.

Thymol is iso-propyl-meta-cresol:



If all the thymol were absorbed and if all the thymol were conjugated with sulfuric acid and none with glycuronic acid, the 0.5 gram of thymol would be excreted as 0.7666 gms. of thymol sulfuric acid. This would cause a marked increase in the percentage of ethereal sulfates. If the liver were not functionating properly, the thymol would not be conjugated, and the percentage of ethereal sulfates would be only slightly different from what it had been on the first two days.

One objection to the study of the function of any organ as an index of disease of that organ, is, that it is perhaps possible for the healthy part of the diseased organ to compensate and assume the work of the whole gland. In such a condition of course the functional output of the organ may be normal, and would be no index of the pathological anatomy of the organ. Under these circumstances only marked destructive changes would leave their impress on the functional activity of the organ.

ETHEREAL SULFATE ELIMINATION BEFORE AND AFTER THYMOL ADMINISTRATION.

Case No.	Diagnosis.	Total Sulfur gms.		Ethereal Sulfate Sulfur gms.		Ethereal Sulfate Sulfur % of Total Sulfur.	
		Before Thymol.	After Thymol.	Before Thymol.	After Thymol.	Before Thymol.	Thymol. After
1	Normal	2.0375	2.1295	0.2893	0.5646	14.2	26.8
2	Gastritis	1.9428	1.7427	0.1457	0.3380	7.5	19.4
3	Fracture	2.7467	2.5527	0.3131	0.6024	11.4	23.6
4	Congestion of liver	0.9852	1.0734	0.1753	0.7069	17.8	28.6
5	"	1.7345	1.6982	0.2480	0.3610	14.3	21.2
6	Gall-stones	2.7628	2.8075	0.7597	1.0303	27.5	36.7
7	" "	3.0042	2.6826	0.3965	0.8474	13.2	29.4
8	Cholecystitis	2.7807	2.6437	0.4866	0.7428	17.5	28.1
9	Atrophic cirrhosis	2.2328	2.3029	0.2791	0.3400	12.5	15.2
10	Tumor of liver	1.9492	1.8757	0.1637	0.3676	8.4	19.6
11	Cancer of liver	2.7526	2.6278	0.6083	0.6648	22.1	25.3
12	Syphilis of liver	2.8104	2.9075	0.3990	0.5437	14.2	18.7

It has been our experience, however, that disturbances in the structure of the liver goes hand in hand with disturbances of function, especially as is indicated by sulfuric acid conjugation of the aromatic radicals. We have found that in cirrhosis of the liver the conjugation of thymol with sulfuric acid does not take place to as marked an extent as in the normal state. This question is now being more fully investigated, and in the very near future we hope to make a more extensive report. Meanwhile, we have cited a few cases above.

It will be observed that in the nonhepatic diseases, and in the nondestructive diseases of the liver, a marked increase in the excretion of ethereal sulfates was observed on the day after the thymol administration. In diseases of the liver, like atrophic cirrhosis, cancer of the liver, or syphilis of liver, this organ has lost its power to conjugate the thymol with sulfuric acid. Case number 10 was a benign tumor of the liver, and it seems no destructive changes went on in the hepatic tissue. This case was in the service of Dr. E. B. Haworth.

We hope to study this reaction more fully in experimental hepatitis, if possible.

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SOME TECHNICAL DIFFICULTIES INVOLVED IN THE COMPARISON OF THE DIAZO AND UROCHROMOGEN TESTS*

By Joseph E. Pottenger, M.D., Monrovia, Calif.

SINCE the publication of Weisz'[1] urochromogen test an abundant literature has developed, a very striking feature of which is the wide difference of opinion as to the relative value of the diazo and urochromogen reactions. Weisz found the urochromogen reaction more frequent than the diazo and recommended it as a more sensitive substitute for the latter. He did not give any tabulated comparisons of the two tests. Later workers have tabulated their experiences. Heflebower[2] found the diazo positive in only 29.1 per cent of a series of 72 cases, while the urochromogen was positive in 66.6 per cent. Gullbring,[3] in a series of 168 cases, found the diazo positive in but 28, while the urochromogen was positive in 88. Sinclair[4] reported 148 cases of which only 30 gave the diazo, but 85 gave the urochromogen. Schaeffle's[5] series of 190 cases showed a much closer relation between the two tests,—40 giving diazos, and 51 giving urochromogens. Other workers have reported values between these extremes.

The differences in percentage incidence of diazo and urochromogen in the above reports, indicate either a striking selection of certain types of tuberculosis among the institutions from which the reports were made, or a lack of a uniform technic and criteria for deciding the presence or absence of the reactions.

My early experiences failed to show such remarkable sensitiveness of the urochromogen as compared with the diazo, as was reported by the first group of workers, and it seemed that this difference might be explained, at least in part to variations in technic, due to the rather indefinite instructions of Weisz. His simple instructions are: Dilute the clear urine with two volumes of water and add three drops of a solution of potassium permanganate (1:1000). A yellow color indicates the presence of the urochromogen body. These instructions were considered sufficient,—in marked contrast to the care necessary for performing the diazo test.

Various workers have attempted to standardize certain features of the technic, thus Heflebower used 1 c.c. urine and 2 c.c. of water. Schaeffle "recognized as a positive reaction only a distinct canary yellow which remained fairly permanent, although there were frequent slightly yellow reactions which soon disappeared." Heflebower on this point said: "The appearance of a yellow color shows that urochromogen is present." With these, and a few other exceptions, however, no serious attempt appears to have been made to standardize the urochromogen test. Most workers have been silent on the details of technic.

According to Weisz' instructions, it is evident that in practice two workers might employ widely different proportions of reagent and urine in perform-

*From the Pottenger Sanatorium for Diseases of the Lungs and Throat, Monrovia, Calif.

ing the test,—three drops ranging anywhere from 3/15 to 3/30 c.c. and the quantity of urine used from 3 c.c. to 6 or 7 c.c. In this manner, one worker, by adding 3/30 c.c. of reagent to 6 or 7 c.c. of urine would employ less than 1/4 the proportion of reagent to urine as the other worker who adds 3/15 c.c. of reagent to 3 c.c. of urine.

The following method was employed to determine, first, the differences in color tone and permanency of reaction due to various proportions of urine and reagent; second, the relation existing between the color tones of foam and solution in the diazo and the urochromogen; third, the influence of the age of the specimen upon the test; and fourth, the conditions for the standardization, if found advisable, of the urochromogen reaction. The regular twenty-four hour specimens from patients, and fresh morning specimens from both patients and normal persons were examined. At least one normal urine was placed among the urines to be analyzed, the identity of all specimens being concealed until the tests were made. The work here reported has been carried out over a period of one year.

The records were kept in tabular form, of which the following is one.

TABLE I.

DETAILED UROCHROMOGEN RECORD, INDICATING COLOR CHANGES AT DIFFERENT TIME INTERVALS, WITH DIFFERENT PROPORTIONS OF REAGENT USED AND AT DIFFERENT DILUTIONS.

	1/3 dilution.					1/6 dilution.					1/10 dilution.				
	Initial	15 s.	30 s.	60 s.	120 s.	Initial	15 s.	30 s.	60 s.	120 s.	Initial	15 s.	30 s.	60 s.	120 s.
0.25 c.c.	y-br	++	++	++	++	++	br-y	+	+	+	+	+	y-br	+	y?br-	+	br	+	+
0.15 c.c.	y-r	++	++	++	++	++	y	+	+	br-y +	+	+	br-y	+	lbr-y	+	ly-br	+	lb +
0.10 c.c.	y	+	+	+	+	+	ly	+	+	lbr-y +	+	+	ly	+	+	+	+	+	lbr-y +
0.05 c.c.	ly	+	+	+	+	+	ly	+	+	+	+	+	ly	+	+	+	+	+	lb-y +
	Control br-y ++					lbr-y +					ly-br +								

In making the urochromogen test, 5 c.c. of the 1/3 dilution of urine was placed in each of 5 test tubes, selected so that the column was of uniform depth. In case the test was positive, further dilutions were made, say 1/6, 1/10, 1/15, etc., until the test failed to appear in one or more tubes. Using one tube as control, to the other tubes were added in succession, .25 c.c., .15 c.c., .10 c.c., and .05 c.c. of potassium permanganate (1:1000), by means of a pipette graduated in twentieths. This series corresponds to 5 drops, 3 drops, 2 drops and 1 drop, when 20 drops equals one cubic centimeter. The color change was recorded at once, at the end of 15 seconds, 30 seconds, 60 seconds, and 120 seconds by reading through the end of the tube held against a white background. The colors viewed from the end of the tube are intensified, as compared with the same viewed laterally, and slight reactions will be found by the first method, where they would be considered negative or questionable by the second. On the other hand, where the urochromogen is strong, the appearance viewed from the end is misleading, especially in the 5 drop and 3 drop tubes, the brown tone

predominating; but, if viewed laterally, a deep pure yellow is present. There is no likelihood of confusion, however, on this point. It must be pointed out, however, that my readings contain a more pronounced brownish element than they would, had they been made viewing the tubes laterally. Quantitative differences were indicated roughly by employing the terms, light yellow, yellow, light brown, brown, and their combinations, and by affixing the usual signs | +, +, ++, +++.

The diazo tests were made with solutions according to the original formulæ of Ehrlich. Solution A, Sulphanilic acid 5 gms., Hydrochloric acid 50 c.c., and water 1000 c.c.

Solution B, Sodium nitrite 0.5 per cent. The test solution was made fresh by adding B to A in the proportion of 1:50. Exact quantities of urine and test solution were measured by pipette into a small 3 inch test tube, $\frac{1}{10}$ volume of ammonia was added and shaken. Both foam and solution were described at once. In positive cases the diazo was performed on a series of dilutions of urine in the following table:

TABLE II.

DETAILED DIAZO RECORD ON SAME URINE AS WAS USED IN TABLE I, SHOWING CHANGE IN COLOR TONES DUE TO DIFFERENT PROPORTIONS IN THE URINE-REAGENT MIXTURE.

	Urine Solution.					
½ vol. Reagent + 1 vol. Urine	1 vol. R. + 1 vol. U.	1 vol. R. + 1 vol. U/2	1 vol. R. + 1 vol. U/3	1 vol. R. + 1 vol. U/5	1 vol. R. + 1 vol. U/7	1 vol. R. + 1 vol. U/10
Foam						
L br	Pink	Pink	Pink (?)	White	White	White
Solution						
Red-br	Red	Br-red	Red-br	Red-br	Y-br	Lbry.

Studies were made on 60 fresh morning specimens from apparently normal persons and on 121 from patients, also on 226 twenty-four hour specimens from patients.

TABLE III.

SUMMARY OF UROCHROMOGEN TESTS MADE ON 58 NORMAL SPECIMENS, SHOWING COLOR REACTIONS AND THEIR PERMANENCY IN RELATION TO THE PROPORTION OF REAGENT USED.

Quantity of Reagent	Initial Reaction		15 seconds		30 seconds		60 seconds		120 seconds	
	Yellow	Yellow?	Yellow	Yellow?	Yellow	Yellow?	Yellow	Yellow?	Yellow	Yellow?
0.25 c.c.	39	5	5	5	1	1				
0.15 c.c.	38	2	2	2	1	0				
0.10 c.c.	19	4	1	0						
0.05 c.c.	8	9								

In two of the above urines with specific gravity of 1011 and 1006, the 5 drop tubes contained permanganate in excess, which gave a purple color, persisting for 8 or 10 seconds. The complete absence of yellow in all tubes was obtained in but 18 specimens.

The preceding table shows that yellow reactions occur in the majority of apparently normal urines, a few of which persist for some time depending upon the amount of reagent used. These normal, or pseudo reactions probably depend upon the presence of oxidizable substances other than the urochromogen body, from which, in practice, they cannot be differentiated by color tone. As, however, they are more or less transient, passing into a pure brown of varying degrees of intensity, the time of their duration is very important in excluding them from the typical urochromogen reaction which is much more permanent even when very slight. Since the yellow had disappeared in 30 seconds in all but one and possibly two of the above specimens, special attention was paid to the history of the persons furnishing them. No reason could be found for excluding them from the table.

In order, then, to exclude normal reactions, I have determined on the following standards of time for observation. Five and three drop tubes, 60 seconds; two drop tube 30 seconds; one drop tube 15 seconds.

TABLE IV.

SUMMARY OF COLOR REACTIONS IN FOAM AND SOLUTION OF DIAZO TESTS IN 60 NORMAL URINES.

	Foam.	Solution.
Colorless	48	
Yellow		40
Brown	12	18
Reddish?-brown		2

Two of the above urines giving questionable reddish tones in the diazo solution were excluded from Table III, although, in neither, did the yellow persist to 30 seconds in the urochromogen test. I have never seen a red tone, or even a suggestion of red in a diazo solution in a strictly normal person. One of these persons had been drinking heavily, though otherwise apparently healthy; the other had had tuberculosis years ago, with apparent recovery, and of late years has suffered from chronic constipation, and has shown at all times considerable anemia.

TABLE V.

SUMMARY OF UROCHROMOGEN REACTION IN 167 TWENTY-FOUR HOUR SPECIMENS AND 94 FRESH MORNING SPECIMENS FROM PATIENTS WHO DID NOT SHOW A RED COLOR IN EITHER FOAM OR SOLUTION OF THE DIAZO TEST.

Quantity of $KMNO_4$	Initial Reaction.		15 sec.		30 sec.		60 sec.		120 sec.	
	Y	Y?	Y	Y?	Y	Y?	Y	Y?	Y	Y?
0.25 c.c.	146	91	11	28	1	2				
0.15 c.c.	141	38	6	21		1				
0.10 c.c.	126	32	5	8	1		1		1	
0.05 c.c.	44	43	3	3	2		2		1	

Five drops was found to be excessive in 17 of the tests made. Fifty-three did not show yellow at all in any of the tubes. The results on both twenty-four hour and fresh specimens are combined, as no essential difference could be determined between the separate tables. The twenty-four hour specimens, however, gave a larger proportion of questionable reactions.

Comparing the above with Table III no essential difference is evident in the 5 drop and 3 drop series. The 2 drop series in the above shows a striking increase in initial reactions. The 2 drop and 1 drop series show a slight tendency to persist beyond the time standard determined from Table III. The 2 drop tubes show 1 positive urochromogen and the 1 drop, 2 positive urochromogens, which the 5 drop and 3 drop tubes fail to show. From this it is clear that 5 drops and 3 drops are excessive, resulting in a rapid change to brown. The action of excess of reagent is further shown in Table I, dilution 1/10, where the 5 drop and 3 drop series become rapidly brown, while the 2 drop and 1 drop series give a slight initial yellow developing in intensity to 15 seconds, and then persisting almost without change for 2 minutes. The result is a negative reaction in the 5 drop and 3 drop tubes, although the 2 drop and 1 drop tubes give a splendid positive reaction. This result occurs invariably where a urine giving a positive test is sufficiently diluted.

It is evident then that 5 drops and 3 drops of reagent to 5 c.c. of dilute urine is not the optimum proportion in which to make the urochromogen test. Summarizing the objections to these quantities we find,—(1) they are excessive, particularly the former, for some urines, imparting the purple color of the reagent to the mixture; (2) they intensify the initial reactions and increase the difficulty in differentiating from normal reactions; and, (3) slight reactions are overlooked due to rapid development of brown color tones.

It is interesting in this connection that Heflebower in the article cited, used his reagent—urine mixture in the same proportion as the 5 drop—5 c.c. urine mixture above. He used a 3 drop—3 c.c. mixture. Of the many workers who have published reports, he has given the most complete statement of technic.

While it is possible that Weisz and others following him have made the reagent-urine mixture in the optimum proportions, i. e., 1 to 2 drops to 5 c.c., his original statement of technic is inadequate; and, in order to guard against error in technic and interpretation, the technic should be standardized.

STANDARDIZATION OF THE TECHNIC FOR THE UROCHROMOGEN REACTION.

Put 5 c.c. of 1/3 dilution of urine (1 part U. to 2 parts water) into each of two test tubes, selected so that the column of liquid is 3.5 cm. deep. Hold over a strong white background and place 0.1 c.c. potassium permanganate (1:1000) in one of the tubes. Holding the tubes vertically, and looking into them from the end, record any increase of yellow at the end of 30 seconds as a positive urochromogen reaction.

In the following table D+ is a positive test with pink foam. D+? is a questionable reaction, in which the color is suggestive of pink, but uncertain. In practice, it has been my custom to call D+? reactions "suggestions" and almost invariably when found, if an afternoon specimen is called for from the same patient a distinct diazo will be found. U+? is a questionable urochromogen reaction. Although a red or reddish solution is not accepted as a proper criterion for determining the presence of the diazo, because of the fact that many known substances and some drugs are known to give similar reactions with the reagent, I have had no reason to suspect confusion on this point, and while I

have accepted the standard criteria in determining the presence of a diazo, I have long suspected that we have missed much that the reaction offers us because we have failed to observe the color tone of both foam and solution. My experience with the diazo reaction extends over eight years, having found it about 2,000 times in 8,800 twenty-four hour specimens from approximately 1,500 tuberculous patients.

TABLE VI.

COMPLETE SUMMARY OF DIAZO AND UROCHROMOGEN TESTS FROM 738 SPECIMENS INCLUDING 391 IN WHICH ONLY THE 5 DROP AND 3 DROP PROPORTIONS WERE USED, FROM 183 PATIENTS.

Color of the Diazo Solution	D−	D+	D+?	U−	U+	U+?
Red	16				16	
	3					3
	4			4		
		115			115	
		2				2
		2		2		
			9		9	
			1			1
			4	4		
Red ?	9			9		
	3				3	
	1					1
Deep brownish yellow	2				2	
	1					1
Yellow	2					2
	1				1	
Canary	1					1
Totals:	43	119	14	19	146	11
D− and D+? transferred to D+	20	156	0	19	146	11

The table gives 119 diazos and 146 urochromogen reactions. But, if we add to the positive diazos, the 23 D— reactions with a distinct red tone in the solution, we have a total of 156 reactions which would be balanced by the addition of questionable urochromogen reactions, making 157. However, there are still 13 abnormal diazo reactions in which a questionable reddish solution was found, the predominant tone being brown or yellow, or both. The foam in these cases was usually a much deeper brown than is found in normal reactions. If these thirteen abnormal reactions are added to the 156 reactions, we have a total of 169 abnormal diazo reactions, as compared with 157 urochromogen reactions.

In making the diazo and urochromogen tests on various dilutions of urine, illustrated by Tables I and II, another source of confusion in comparing the tests was discovered. In several instances, with a positive diazo, the urochromogen was recorded either negative or questionable at 1/3 dilution, while dilutions of 1/5, 1/6, etc., gave unquestionable reactions. There were four

specimens, all deep yellow in color, giving U— reactions, at 1/3 dilution, but at 1/6 dilution two of them were distinctly positive and the other two were questionable in the 2 drop and 1 drop tubes. This difficulty was met with only in concentrated, particularly deep yellow urines. Brownish urines gave no trouble on this point. From this it is clear that some urines should be diluted more than 1/3 as Weisz recommended, in order to demonstrate the reaction.

Comparisons were made between tests on fresh specimens and on the same specimens after twenty-four hours standing at room temperature. No important differences were found between the amounts of the diazo and urochromogen bodies in the two examinations. Certain urines, however, giving U— reactions when fresh, gave U+? reaction at the end of the twenty-four hours. This was attributed to the development of brown color in the specimen, due to light, so that extremely slight reactions would be noticed, which were overlooked in the fresh yellow specimens.

CONCLUSIONS.

1. The remarkable sensitiveness of the urochromogen reaction as compared with the diazo reaction, reported by some workers, is due in large part to failure to exclude the normal transient reactions found in normal urines and to a hesitancy in recording slight diazo reactions.

2. The urochromogen is somewhat more sensitive than the diazo if the pink foam alone is considered in determining the presence of the latter; it is equally sensitive to the diazo if, compared with all reddish reactions in the solution of the latter, and less sensitive than the diazo if all questionable reddish solution reactions and those with deep brown foam are added to the latter.

3. The difference in color tone and permanency of reactions affected by various proportions of reagent and urine in performing the urochromogen test makes it imperative that the test be standardized.

4. The diazo reaction, if studied carefully as to color tone of both foam and solution, will give us considerably more information than the urochromogen gives.

5. In view of the prevailing confusion in the matter of technic, it seems too early to draw conclusions as to the relative prognostic values of the two reactions.

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CASES OF INFECTION TERMINATING IN SEPTICEMIA*

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ONE has always to differentiate between primary and secondary infections, just as one must recognize localized and generalized (*septicemic*) infections. One may take as a type of severe primary infection erysipelas which may or may not be septicemic. So long as it is localized, even though it is spreading, it is not a particularly serious affair, but let the organisms gain entrance to the blood stream, and it becomes a very serious menace to the life of the individual. Erysipelas may also be secondary, but the usual cases one sees are primary in the usual sense of the word. On the other hand, one may consider a case of lobular pneumonia appearing as a sequel of diphtheria. The pneumonia may be the result of lowered resistance caused by the attack of diphtheria, or it may be the result of aspiration of materials from the larynx during a period of partial asphyxia caused by the presence of the laryngeal or tracheal membrane. In either case the pneumonia is secondary whether or not it be caused by the diphtheria bacillus or some other more frequent pulmonary invader.

Again we recall the frequent terminations seen in connection with cases of nephritis in which the final stages of the life of the patient are marked by appearance of friction rubs in evidence of pleuritis or pericarditis, or the intestinal upsets indicative of the too frequent pseudomembranous enteritis. Here we are dealing with what has been called a *terminal infection* which, in the reduced state of the patient's resistance, overwhelms him and hastily carries him off. Again, when in case two or more organisms appear in a lesion we speak of *mixed infection* by which we often mean that we are not able to determine which of the germs present was the primary invader.

The following histories illustrate well a certain type of infection and also they illustrate well the organic and tissue changes produced by two organisms, streptococci and staphylococci, in septicemia.

CASE I.

The first example to be offered exemplifies the type of severe, essentially primary, streptococcus infection, which became a mixed infection later, but which evidently was the immediate cause of death. In this case, as is frequently the case, there was no demonstrable localization of the organisms which, while they possibly had their nidus in the mouth or pharynx, gave no definite indication of such a localization but proceeded to grow rapidly and invade the blood stream by which they were generally distributed.

A. R., Hospital No. 1005, was admitted to the Cincinnati General Hospital on April 6, 1915. He died on April 12 of the same year.

Clinical Notes.

Complaint.—Pain, which at times is sharp, in the epigastrium and in both hypochondriac regions.

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Family History.—Irrelevant.

Past History.—The patient stated that he had not had the common diseases of childhood. Seven years before admission he had been operated upon for a left inguinal hernia. He denied venereal disease, but said he smoked and chewed tobacco and drank beer and whisky to excess until three months before admission.

Present Illness.—Nine days before admission he ran up a flight of stairs and became suddenly dyspneic. He went home and shortly thereafter developed the pain of which he complained. For seven days he had been aware of the rapid beating of his heart. For this he had seen two physicians who told him to go to bed and remain there. He was not able to have proper care at home and came to the hospital.

Present Condition.—The patient was a white man, young, well-developed and well-nourished. On entrance his temperature was 98.6, pulse 168, respiration 48. He was dyspneic, had a very rapid heart, and pain in the upper part of the abdomen. The pupils reacted to light and during accommodation. The conjunctivæ were clear. His teeth were part false; those remaining, and the gums, were in poor condition and evidently neglected. The neck was not evidently abnormal. The chest was well developed; the right side was more prominent than the left. Anteriorly the percussion note was somewhat hyperresonant; posteriorly, at the right base, the resonance was diminished, and the tactile fremitus reduced. Auscultation showed that the breath sounds were roughened, with piping râles over the left, and the mucous râles from the scapular angle to the base on both sides posteriorly. The heart was evidently enlarged. Dullness was marked in the right second interspace, and the whole right border of the heart extended beyond the normal line. Also, the area of cardiac dullness extended to the line of the left nipple. The heart rate was rapid (168) but the rhythm was regular, and at the systolic orifice there was evidence of roughening of the sounds. The lower border of the liver was palpable below the costal margin. There was no abdominal tenderness, distension or rigidity. In the left inguinal region was the scar remaining from the old herniotomy. There was no edema of the extremities. The genitals were not abnormal. Upon the back there were a number of macules showing a tendency to become papular.

The third day after admission (April 9) the patient was cyanotic, and the apex beat was plainly visible. On auscultation moist râles were heard everywhere, especially over the left lung. The pulse rate was 156. Ten ounces of blood were let by venesection. The following day the cyanosis was less evident, and there were numerous hyaline casts in the urine. On the next day the general condition was worse; there was edema of the lower extremities, severe cyanosis. The urine showed abundant hyaline casts. On the following morning the patient died.

Clinical Diagnosis.—Acute cardiac dilatation; mitral insufficiency; embryocardia; acute nephritis.

Autopsy Protocol.

The body was that of a young adult, well-built and well-nourished white man, 5 feet, 8 inches long, and apparently about 30 years of age. He had a scanty beard. The pupils were equally and moderately dilated. Rigor mortis was present, and postmortem lividity was vivid. The whole body, especially the upper half was tremendously cyanotic, and scattered over the body, but especially over the thorax, particularly over the back, were numerous small hemorrhagic lesions, most of them drying and crusted, which in some respects resembled the lesions of a malignant scarlet fever. On the outer surface of the right arm, at a point about the middle, was a subcutaneous nodular mass that had something the appearance and feel of a lipoma, but which seemed to fluctuate, and which, on section, proved to be an abscess filled with a thick greenish-yellow pus. Smears showed staphylococci present and cultures proved these to be the Staphylococcus pyogenes albus. When the body was turned on the side, a large amount of clear, sanguineous fluid ran from the nose and mouth, and this was followed by a clear thin mucoid fluid containing material which looked like coffee grounds.

The face had a generally swollen appearance as had the whole body, and this was especially marked in the neck. There seemed to be evidence, to the eye, of a general edema, and yet there was very little pitting on pressure but only local pallor. The neck and the back of the head, including the ears, were a deep purple color. On the inner aspect of the left elbow was a wound caused by a hypodermic needle. Seven cm. medianly from the left nipple was another punctured wound (hypodermic) which led to an area of hemorrhage in the great pectoral muscle. There was the slightest edema of the legs. The peripheral lymph glands were palpable but not enlarged remarkably.

The median section showed that the muscles and subcutaneous tissues were exceedingly edematous and that the greenish colored fat was the seat of a serous infiltration. In the abdomen there were 600 c.c. of a cloudy serous fluid. The diaphragm reached to the lower edge of the 6th rib on the left and to the upper border of the 6th rib on the right. Both pleural cavities were filled with a clear sero-sanguineous fluid. Just beneath the ensiform and reaching a centimeter or more below it, was a mass of tissue which in general structure resembled jaundiced salivary gland tissue. Part of it also resembled to the eye, lymph glandular tissue. Upon investigation it was found that this tissue was a part of a mass of tissue which ran upward into the thymus region and that it was continuous with what seemed to be the thymus itself. In the thymus region there was no distinct line to be seen between thymus tissue and adiposa, (because of hemolytic changes) but about the trachea and in the upper mediastinum, this tissue formed a mass that weighed perhaps 50 grams. From it there ran down over the pericardium, a wide, thin mass of the same tissue, which divided at about the upper end of the area of absolute cardiac dullness to run laterally on either side, parallel to and just beneath the median edges of the lungs to the diaphragm when again the volume of the tissue was increased. This tissue and all the mediastinal tissues were exceedingly edematous and gelatinous in appearance. In the thymus-like material which completely surrounded the trachea just above the bifurcation, the tissue was infiltrated diffusely with a thin sero-purulent fluid which contained capsulated Gram-positive diplococci, short chains of streptococci and small clumps of staphylococci. There were no fusiform organisms nor spirochetes present. The trachea, larynx, tongue and tonsils were removed *en masse*, and except for edema of the perilaryngeal tissues, showed nothing except an intense congestion and cyanosis. This was so severe that the thyroid appeared to be the seat of an acute inflammation. The peritracheal and bronchial glands were hyperplastic and moist. The tonsils showed no evident abnormality other than atrophy. The teeth were not exceedingly bad, but there were some bridges and the upper incisors were false.

When the thorax was opened, the lungs did not collapse. Over the middle third of the upper left lobe, on the pleura, was an area measuring roughly 4.5 cm. in diameter, which was congested and the surface of which was covered with a scanty recent fibrinous exudate. Again, over the middle third of the anterior half of the left upper lobe was a similar but less definitely defined area of the same character. The upper four-fifths of the left upper lobe was almost completely consolidated, and on section had the appearance of a massive red hepatization modified by unusual edema, and hemolysis. The blood vessels leading to this area were all patent. Except that the process was less advanced, the right lung showed the same general appearance. (Weights, left lung, 1750; right, 1450.)

The pericardium contained an increase of cloudy serous fluid. There was a large edematous milk spot over the left ventricle near the apex, and a small one in the usual place over the right ventricle. In the pericardium over the base, were very numerous petechiæ. The heart was large, but was not weighed. The myocardium was only moderately hypertrophied but the cavities were much dilated. The orifices also were dilated and in this the pulmonary participated. The myocardium was pale and evidently the seat of fragmentation. The valves were not abnormal. There was no essential thickening and no vegetation on any leaflet or segment. The aorta was smooth and not sclerotic. The endocardium, including the valves and the aortic intima, was stained with the products of hemolysis. Between the columnæ carneæ of the left ventricle were numerous globular mural thrombi all of them with softened grumous centers.

The spleen (230 grms.) was adherent by old adhesions to all the surrounding organs. It was slightly enlarged, firm, moderately edematous, uniformly grayish-brownish red in color, not softened and the glomeruli were not distinct.

The liver (2116 grms.) was enlarged. The anterior surface showed a well marked capsular thickening, so diffuse that only occasionally could one see the color of the liver substance through small clefts in the hyaline membrane. On cross section the organ showed a typical nutmeg appearance.

The pancreas was slightly congested but otherwise not apparently abnormal.

The kidneys were large (right, 225 grms.; left, 295) and moderately congested and extremely cloudy. The capsules removed with ease for the most part, though the cortex was torn in a few places. The stellate veins were only occasionally dilated. The line of demarkation between cortex and medulla was only moderately distinct. The cortex and medulla were both congested, the medulla rather more so. The glomeruli were not distinct. The cortex was thickened (9 mm.) The pelvis and ureters were normal.

The adrenals showed nothing remarkable other than congestion. They were of normal size.

The mesentery was quite edematous and on section had somewhat the same appearance as the mediastinal (thymus?) tissue. The lymph glands were pale and juicy, but not hyperplastic.

The large bowel was filled with firm, rounded fecal masses. The appendix appeared to be normal. There was no abnormality of position of the abdominal organs. The stomach was filled with a large amount of thin fluid, dark grayish-green in color, in which were large solid coagula of milk. There was a moderate grade of chronic catarrhal gastritis evident. The gall ducts were patent. The papilla of Vater was apparently normal. The gall bladder contained a fair amount (50 c.c.) of a mucoid brownish-green bile with no stones or sand.

Nothing abnormal was observed in the small intestines, but in the large bowel the lymph follicles were diffusely enlarged and congested.

The genital tract seemed normal.

The brain (1625) showed intense congestion of the pial vessels together with a marked edema. There was no evidence of meningitis. The spinal fluid was increased in volume. There were no other lesions observable. The pituitary appeared to be normal.

Anatomic Diagnosis.—Cervical (peritracheal) and upper mediastinal cellulitis; Septicemia; Generalized edema; Cardiac hypertrophy; Cardiac thrombi; Cardiac dilatation; Acute pseudolobar pneumonia; Pulmonary edema; Splenic and hepatic hyaloserositis; Passive congestion of the liver, lungs, kidneys, stomach, intestines and spleen; Acute pleuritis; Abdominal pleural and pericardial effusion; Chronic and acute diffuse nephritis.

In this case we seem to see an illustration of the malignant course of an infection which progressed without any definite localizing onset. The clinical history is brief, to be sure, and perhaps imperfect. There is no history of sore throat nor of pain in the neck. The teeth were in a foul condition beneath the bridges. The infection commenced insidiously, causing cyanosis and epigastric pain. Even at this time the myocardium was evidently damaged, and then later came the evidences of pulmonary and renal involvement. The main organism in this was evidently the streptococcus longus or hemolyticus. The staphylococcus was a secondary invader, possibly from the skin lesion on the arm, and the gram-positive diplococci possibly of pulmonary origin, and may be looked upon as producing a terminal infection.

CASE II.

This case is a typical one due to pure infection with the Streptococcus hemolyticus.

Clinical Notes.

W. P., a white man aged 71, was admitted to the Cincinnati Hospital on July 8, 1916. He died on July 10, 1916. At the time of admission he had a normal temperature, though the face was marked with the erythema of early erysipelas. He gave a history of having been drinking a good deal. At the time of admission his lungs were clear. The heart rate was increased, and the heart was slightly enlarged. There was a definite accentuation of the second mitral sound. That afternoon and the following day the temperature became elevated, and there was some slight roughening of the breath sounds over the upper part of the left lung, but no râles were heard. During the day the temperature remained high and the patient became restless. At the same time the face become more swollen and the inflammation appeared to be extending to the neck which showed some erythema. On the following day at 8 o'clock a. m., the temperature was 101.8 F., the respirations were increased, the neck was swollen and the inflammation had extended toward the thorax. At this time the patient commenced to have difficulty in swallowing. Also, the respiratory sounds over the upper left chest were more roughened and there were a few moist râles. At 1:30 p. m. the patient appeared cyanotic, respiration was quickened and the pulse was

very rapid and compressible. Large moist râles were heard over both lungs but especially over the left. In spite of stimulation, temperature, pulse and respirations rose, and the patient became progressively weaker and died at 8 p. m.

The *Clinical Diagnosis* was erysipelas.

AUTOPSY PROTOCOL (M. C.).

The body was that of a slenderly built, fairly well nourished white man apparently about 50 years old. Rigor mortis and posterior lividity were present. The face, neck and scalp were tremendously swollen. The eyelids were swollen shut and the surface of each lid presented a superficial excoriation which extended along the margin. The pupils were equal, there was a beginning cataract in each eye and a well marked arcus senilis. All the tissues in the region of the face and neck felt boardlike, the skin was excoriated and, especially over the anterior surface of the neck, the superficial layer of epidermis was entirely missing. There was a diffuse reddish-purple discoloration over the entire face and neck. This boardlike thickening and discoloration involved the mouth on the right side. The teeth were almost all gone and those which remained were carious and necrotic. The gums were in a fair condition. The appendix was *in situ*, lying free directly behind the cecum. There was nothing unusual about the arrangement of the intestines and no evidence of any inflammatory process in the peritoneal cavity. The omentum extended like an apron across the upper abdomen.

When the thorax was removed, the lungs did not collapse. Both pleural cavities, more particularly the left, were almost completely obliterated by dense fibrous adhesions everywhere. In the pericardial sac there was about 50 c.c. of a clear, straw-colored fluid.

When the calvarium was removed, the dura was not unusually adherent. There was an increase in cerebrospinal fluid which, however, was clear. The surface of the brain was very edematous and the vessels were all congested and engorged. The vessels at the base of the brain were markedly sclerosed. There were some old adhesions between the upper lobe and the diaphragm.

The heart was slightly larger than normal and dilated on the right side. The mitral valve, except for a few areas of fatty degeneration at the base, was apparently competent and healthy. The tricuspid and pulmonary valves were apparently healthy. There were a few calcareous placques in the sinuses of Valsalva near the base of the aortic valve, but the leaflets were thin and the valve apparently competent. There was a well marked fibrosis of the papillary muscles and of the mural myocardium. The aorta was remarkably healthy for the age of the individual and was marked only here and there by areas of fatty change.

The right lung was rather voluminous and felt boggy although it crepitated in its entirety. There were the tags of many old adhesions throughout the pleura and beneath the pleura were numerous shotlike areas which evidently represented calcareous obsolescent tubercles. The cut surface was moist and in the dependent portions bright red and congested. There were no areas of consolidation. The left lung was almost completely destroyed in removing it. Throughout the substance of the organ one could feel very numerous obsolescent tubercles. Otherwise the tissue was merely edematous and congested.

The liver was of fair size and felt rather firm. There were old adhesions between the diaphragm and the upper surface of the right lobe which was scarred. The entire capsule was diffusely marked with fibrotic lines. There was a groove running longitudinally in the middle of the ventral surface of the right lobe, which was 2 cm. deep and 9 cm. long. On cross section, the organ appeared cloudy and was only slightly congested. The gall bladder contained about 20 c.c. of rather sticky, brownish-yellow mucoid bile, and no calculi. The mucosa was apparently thickened.

The right kidney was about normal in size. The capsule split in removing and when finally removed left a surface which was more or less torn and roughened. The organ was very firm to the feel. On cross section, the cortex was somewhat thinner than normal. The glomeruli and interlobular vessels were slightly congested and there was a diffuse fibrosis throughout the entire organ. The left kidney was slightly larger than the right. There were several retracted areas which were purple in color upon the outer surface of the cortex, otherwise the organ resembled the right.

The spleen was small, slate color, and on section showed nothing abnormal.

There were no noticeable lesions in the other organs.

Anatomic Diagnosis.—Diffuse cellulitis of the head and neck (erysipelas); Edema and congestion of the brain; Hypertrophy and dilatation of the heart; Fibrosis of the myocardium; Chronic diffuse nephritis (arteriosclerotic type); Peripheral arteriosclerosis; Pleural adhesions; Obsolescent pulmonary tuberculosis; Edema and congestion of the lungs.

In this case we have an example of an infection severe in degree and rapidly becoming septicemic. In this case, as in the previous one, the starting point was not discovered, and, in fact, it not infrequently happens in erysipelas that the portal of infection remains hidden. It is possible that the disease originated from the teeth, or, it may be, that it came from one of the nasal cavities. A general discussion of the origin of this disease has been given by Holmes.[1] For the rest, it is remarkable how little visible general organic damage is caused in these cases. Certainly the heart was poisoned—the dilatation is evidence of that, but aside from a rather moderate edema there was little change in the other tissue which could be attached to the streptococcic infection.

In a sense this case represents also one of terminal infection in an individual weakened by alcohol. It is in such cases that erysipelas is most fatal.

CASE III.

This case exemplifies the occasional fate of the body in staphylococcus infections.

Clinical Notes.

A. G., a white boy, 10 years old, was admitted to the Cincinnati General Hospital on July 2, 1916. He died the following day. The following meager history was obtained:

At the age of 3 years he had had "hip joint disease," by which was meant that one of his legs appeared short and painful, and produced a limp. This lasted one month. At the age of 9, he had measles. During the past year he was struck in the abdomen by a swing, since which accident he has complained of burning pain on urination. Also since he was ten years old he was struck by a motor cycle, receiving a slight scalp wound. He has a history of being awkward, of stumbling over and bumping into things.

Six days before admission while at the playground, he was pushed into the water. He lit on his feet. The following day he was somewhat lame. The next day the pain in the hip was severe and the leg was swollen. Two days later he was delirious, so his people said, and a physician was called. During that night and the next day the thigh became reddened and superficial vesicles appeared over it.

When he was admitted he had not urinated for 24 hours and his bowels had not moved for four days. No other data were given in the clinical history.

Autopsy Protocol (J. S.).

The body was that of a fairly well developed, fairly well nourished, white boy, apparently 14 years of age. Postmortem rigidity was present in the legs but was passing away in the arms. Lividity was well marked over the dependent portions of the back. At the outer angle of the right eye and beneath the right eye, there was some subcutaneous extravasated blood. The pupils were equal and neither dilated nor contracted. On the whole anterior surface of the right thigh and the left elbow, there were some small ecchymotic areas. Over the right elbow there was a small superficial abrasion. The right leg from Poupart's ligament to about 2 inches above the knee was larger than the left and on the external aspect of the thigh, 2 inches below the anterior superior spine, were numerous (about 5) vesicles containing a clear fluid. The abdomen was slightly distended. There was very little subcutaneous fat but the muscles were well developed.

When the sternum was removed, the lungs partially collapsed. There was no excess of pleural fluid. The pericardial fluid was slightly increased, was cloudy and straw-colored and contained small floccules of fibrin. The intestines were distended with gas. The appendix was *in situ*. The peritoneum was smooth. The mesenteric glands were slightly enlarged to about the size of a bean.

On the outer lateral aspect of the right lung, there was a thin sero-fibrinous exudate, slightly adherent. On the corresponding area on the parietal pleura there was a similar exudate. Over the lateral border of the upper lobe there was a small area which did not

[1]Holmes: cf. MacCallum, Textbook of Pathology, Phila., 1916, p. 492.

crepitate and in which there was a small abscess. The tissues surrounding this area were deeply congested. The lower border of the lower lobe was deeply congested, and small pieces sank in water. The upper lobe showed, near the apex, another small abscess the surface of which was covered by a fibrinous exudate. Except for these areas, the lung crepitated throughout and showed merely congestion. The left lung had no fibrinous pleuritic exudate and crepitated throughout except for two or three small shotty areas which on section proved to be obsolescent tubercles. The apex was scarred.

The heart was of normal size and contained upon its surface a fine granular serofibrinous exudate which was slightly adherent and when removed left a granular surface. This exudate was equally distributed over the right heart. The heart was firmly contracted and the various cavities contained mixed clots. All of the valves were healthy. The myocardium was pale but showed no abnormality. The foramen ovale was covered by a thin membrane which could be pushed aside leaving an opening about the size of a lead pencil.

The liver was of normal size, cut easily, the edges turning out. The organ was of a deep red purplish color with mottled yellowish areas. The parenchyma was somewhat congested and showed areas of fatty change.

The spleen was of normal size, its surface perfectly smooth, it cut easily and the cut surface showed well marked Malpighian corpuscles and some slight increase in fibrous tissue.

The kidneys were of normal size, pale in color, cut easily and showed pale surfaces. The cortices were well marked off from the medulla. The glomeruli showed as fine glistening points. Throughout the parenchyma there were scattered fine abscesses varying in size from a pinpoint to 2 mm. in diameter. The capsule stripped with ease, showing deeply congested stellate veins and numerous small subcapsular abscesses.

The brain was deeply congested but not edematous and was preserved in formalin for further section.

The entire gastrointestinal tract was apparently healthy except for a slight congestion in the stomach and duodenum with a few pinpoint submucous hemorrhages near the pylorus.

On section, the medullary cavity of the right femur was deeply congested and inflamed. The process had not as yet gone on to pus formation. The hip joint contained a small amount of thin purulent material.

Anatomic Diagnosis.—Acute osteomyelitis; Acute purulent arthritis; Acute septicopyemia; Acute pulmonary and renal abscesses; Acute fibrinous pleuritis and pericarditis; Cloudy swelling and congestion of the liver; Congestion of the brain; Acute cellulitis of the right thigh; Multiple contusions and abrasions.

In this case a trauma, not of a severe nature, furnished the starting point for the localization of staphylococci which were floating in the blood stream. Evidently these organisms attacked the joint and the medulla of the bone, and, being of a virulent type, rapidly spread to the periarticular and periosseous tissues and produced a well marked cellulitis. At the same time they invaded the blood stream in large numbers and in transit through the organs of the body, many of them became lodged, especially in the lungs and kidneys where they produced typical focal lesions—metastatic abscesses, evidence of septicopyemia, and in the pericardium where they set up an acute inflammation.

CASE IV.

This case illustrates the somewhat slow progress of a serious infection terminating in septicemia.

Clinical Notes.

B. T., a negress of 30 years, was admitted to the Cincinnati General Hospital on June 4, 1916. She died six days later. The following history was elicited: Three weeks before admission the patient missed her menstrual period. Four days later she took 10 grams of quinine and a glass of whisky, and then, a day or two later, the flow commenced. During the following five days she passed a few blood clots. Since that time she has had no bleeding, but has had a yellowish discharge from the uterus. Soon after the menstrual flow was re-established she had chills and fever, and her feeling of illness

kept her more or less confined to her bed. About a week before admission she developed pain in both sides of the neck and in the left posterior occipital region, and at that time she also complained of pain "all around the inside of her head." During the three weeks previous to admission she vomited frequently. The day before admission she was attacked by lachrymation in the left eye. The conjunctiva rapidly became bloodshot and before noon the vision was dim. This was accompanied by severe pain.

Previous to this illness the patient had been well. She was married in 1910, and had one child living and well. Her husband was well. She denied venereal disease, but for five years had had a leucorrhœa.

On admission her temperature was 104.8, pulse 104, respirations 36. She was weak, fairly well developed, and not extremely toxic in appearance. The right eye was rather dull, but the pupil reacted to light and during accommodation. The left cornea was bulging, covered with pus and was opaque. The conjunctiva was filled with pus and was congested. The ears and neck showed nothing unusual; the cervical glands were palpable. The tongue was coated, the papillæ slightly enlarged, the breath foul, and there were petechial hemorrhages in the mucosa. The teeth were in bad condition and the gums were pyorrhœic. The tonsils were small.

The thorax was well developed, and resonance and expansion were good. In the left lung there were a few high-pitched râles on expiration.

Examination of the heart showed an even rhythm, but fast rate, and everywhere systolic and diastolic murmurs were heard, with a suggestion of a friction rub. The pulses were equal and regular, but not full.

Nothing noteworthy was discovered in examining the abdomen.

Pelvic examination showed a multiparous perineum. The uterus was enlarged to the size of one at the second month of pregnancy. It was retroverted one degree, and was difficult to outline. The cervix was torn bilaterally, and was somewhat softened. The os was open. The vaults were free and not tender. There was a considerable cheesy discharge from the uterus.

The urine was clear, tea-colored; specific gravity 1005; acid in reaction; no sugar or albumin; a few hyaline and many granular casts; and some pus cells.

On the day following admission, the uterus was cleaned out. The cervix was found closed and in normal position. After dilatation some tissue the size of a prune was removed. The uterus was washed out with sterile water and with sterilizing solution and then packed with iodoform gauze. The tissue from the uterus proved to be almost completely necrotic material with a few remnants of chorionic villi. The blood taken from a vein on the day of the operation gave a negative Widal but gave a pure culture of streptococcus.

The following day the packing was removed and the uterus was irrigated. At this time the patient was very nervous and weak and had a temperature of 105 F. On the next day the right eye showed evidence of ophthalmitis, and the general condition of the patient remained as on the previous day. The next day Cheyne-Stokes breathing was present, and two days later the patient died in coma.

Clinical Diagnosis.—Self-induced abortion; General sepsis; Panophthalmitis (left); Sympathetic conjunctivitis (right); Pelvic peritonitis.

Autopsy Protocol (L. M.).

The body was that of a well nourished, brown-skinned negress about the age of 30. Neither postmortem rigidity nor lividity was present. The pupil of the right eye was dilated. There was evidence of an infection in the deeper structures of the left eye. The cornea was totally destroyed, the eyeball atrophied and there was a slight congestion of the scleral conjunctiva.

The subcutaneous fat was much increased and the skeletal muscles were well developed. On opening the chest, the lungs were somewhat collapsed and there was no free fluid in either pleural sac. The pericardium was free and contained no abnormal amount of fluid. The omentum contained a fair amount of fat, and was adherent by fresh fibrinous adhesions to the distended intestines. Throughout the entire abdomen, the intestines were adherent to the surrounding structures and to each other by fresh, fibrinous adhesions. The spleen was adherent in like manner to the under surface of the diaphragm, as was also the liver. In the cul-de-sac of Douglas, there was an abscess, walled off, containing a greenish-yellow pus.

The right lung, of a bluish-gray color, was free from adhesions and crepitated through-

out. The cut surface was rather pale in appearance, and appeared healthy except for a moderate congestion in the base. The left lung was similar to the right.

The heart was of normal size and surrounded by an increased amount of pericardial fat. The right side was slightly dilated. On one of the leaflets of the mitral valve, there was formed a vegetation and ulceration such that the leaflet was almost destroyed. The remaining valves showed nothing abnormal. The myocardium was pale in appearance, but there was no gross pathological change. The coronary orifices were patent, and the coronaries appeared healthy, as did also the aorta.

The liver was slightly larger than normal, of a reddish hue, and fairly firm in consistence. The organ cut with slightly lessened resistance, and had a cloudy grayish appearance. There was no evident increase in fibrous tissue

The spleen was about twice its normal size, rather soft to the feel, and on its upper surface, beneath the capsule, was a roughly triangular, reddish-blue area, about 2 cm. along its base and 1 cm. along the perpendicular. There were three other such areas scattered beneath the capsule. On section these were found to be in the substance of the spleen, and to be of the same consistence throughout. The cut surface of the spleen was a dark purple color, and very pulpy throughout. The Malpighian bodies were not visible.

The right kidney was of about normal size, and surrounded with a slight increase of perirenal fat. On the outer surface there was an area somewhat similar to those seen in the spleen, and extending likewise into the substance of the kidney. The capsule stripped with fair ease, the surface beneath being smooth and the stellate veins very slightly congested. The cortex was somewhat thicker than usual, of a grayish red color, and the line of demarcation between medulla and cortex was rather indistinct. The glomeruli were not visible, and the pyramids were rather indistinct in outline. The left kidney resembled the right, except that no infarcts were present.

The right tube was increased in diameter and its surface was congested. On opening the tube, it was found to contain a slight amount of greenish-yellow pus, similar to that contained in the abscess in the cul-de-sac of Douglas. The left tube showed a slight amount of congestion, but was otherwise apparently healthy.

The uterus was slightly larger than normal, and rather decreased in firmness. On section, the endometrium was congested and easily removed from the myometrium, the latter being rather paler than usual.

Other than the evidences of recent adhesions to the surface, and a slight congestion of the mucous membrane, the intestines showed no pathologic changes.

Cultures from the eye gave pure culture of streptococci.

Anatomic Diagnosis.—Generalized fibrinous peritonitis; Pelvic abscess; Purulent salpingitis; Endometritis; Acute ulcerative choroido-retinitis; Acute ulcerative endocarditis; Renal and splenic infarcts; Acute splenic tumor; Acute parenchymatous nephritis; Septicemia.

As compared with the case of erysipelas this was a chronic case. Also it was chronic in that it permitted not only the appearance of a septicemia, but also in that time was given for the cocci to establish themselves in other than the primarily infected organ, and to produce destructive lesions. Such lesions were those in the left eye, and in the heart. From the ulcerative valvular lesion emboli were carried to the spleen and kidney and there produced infarcts, which, had the patient lived long, would certainly have formed abscesses.

The thing which these case histories demonstrates best perhaps, is that comparatively slight injuries, as in the case of the boy in Case III, or a mere "lessening of resistance" as in Cases I and II, may make the proper conditions for invasion of the body by organisms which are lying in wait in the skin or, more frequently, within one or another cavity of the body. In each of the four cases, it is entirely possible that the organisms were present within the body awaiting for the proper conditions to be developed to suit their needs. They also demonstrate what serious lesions may be produced by such frequent bacterial inhabitants of the body. One may imagine that in each case the infection might have been somewhat less severe, and that the patients might have

lived. He will then suggest to himself that in the first case the man would have had a somewhat weakened heart, possibly carnified patches in his lungs; possibly peritoneal, pleural and pericardial adhesions,—(in the splenic and hepatic capsular changes there are evidences of an older infection). In the second case he might consider that the patient would become entirely well again save for a slight myocardial defect and a tendency to recurrence of the erysipelas. In the third case, the infection might result in merely a stiff hip joint, by the formation of adhesions; or in a chronic osteomyelitis with all its associations. In the fourth case, apparent recovery would be attended by localization of the pelvic abscess, and encystment of the tubal exudates, not to mention pelvic adhesions between bowel and the pelvic organs. All of these slight ailments are more frequently seen than the severe ones described in the foregoing protocols. Nevertheless both are due to the action of the same organisms the effects of which in the body depend upon the opportunities for growth, by which one means the state of the tissues with respect to their powers of resistance to infection.

LABORATORY METHODS

CLINICAL METHODS FOR DETERMINING THE BUFFER ACTION OF THE BLOOD*

By J. J. R. Macleod, M.B., Cleveland, Ohio.

COMPARISON of the H-ion concentration of normal blood with that of patients in whom for one reason or another an increase of this value might be expected (e.g., high excretion in the urine, presence of "acetone bodies" in urine, the development of symptoms usually attributed to "acidosis") has shown either no change at all or one of trivial magnitude. It has been in grave cases of acidosis alone that definite increase in H-ion concentration has been found present, thus indicating that any distinct change from the normal is incompatible with life. That this is true even when very considerable quantities of acid are being discharged into the blood, as a result of faulty metabolic processes, (e.g., in diabetes) indicates that some mechanism must exist in the blood for taking up the excess of acid without permitting any or only a trivial change in H-ion concentration. Thanks mainly to the work of L. J. Henderson, we now know that this mechanism consists essentially in the ability of phosphates and carbonates each to exist as two types of salts, acid and neutral. When these salts undergo dissociation and hydration, they do so in such a way as to yield a mixture of intermediate salts with different dissociation constants and thus capable of liberating varying quantities of H and OH ions.

Thus, let us compare the effect of adding some mineral acid to water and to a watery solution of the two phosphates (Na_2HPO_4 and NaH_2PO_4) in such proportion that it is exactly neutral to such an indicator as methyl red; whereas a drop of acid suffices in the case of water to produce a marked change in tint, it will be found that considerable quantities of acid have to be added to the solution of phosphates to produce the same change. The presence of the phosphates endows the solution with the power, as it were, of soaking up the excess of H-ions; it acts as a sponge or, less strictly, as a "buffer." In the case of blood it is mainly the salts of carbonic acid and partly the amphoteric property of proteins rather than the salts of phosphoric acid that furnish the buffer.

The above described *chemical* reactions serve to adjust the H-ion concentration temporarily, but when the addition of excess of acid is more persistent, a *physiological* mechanism also comes into play, namely, increased excretion of acid from the body, the volatile acid (CO_2) being excreted by way of the lungs, and the fixed acid, by the kidneys.

These general considerations indicate the importance of determining the buffer action of the blood, and recently two papers have been published which seem to offer simple methods by which this can be done.

*The manuscript for the present article was prepared some months ago, its publication being delayed through error.

THE METHOD OF LEVY AND ROWNTREE.

A test tube made of hard ("nonsol") glass of about 20 c.c. capacity containing about a gram of powdered *neutral* potassium oxalate is filled with newly drawn blood, immediately stoppered and placed on ice. Quantities of 2 c.c. each of the blood are then placed in a series of seven small (nonsol) test tubes and allowed to stand for 5-6 minutes in order to permit a narrow layer of plasma to separate on the surface (this prevents laking of the blood during the subsequent addition of acid or alkali). The blood in the first tube is used for the determination of the normal H-ion. In each of the next three tubes are added respectively 0.1, 0.2 and 0.3 c.c. N/50 HCl, and to the last three, similar quantities of N/50 NaOH. After inverting the tubes so as to mix the contents, the blood in each is transferred to celloidin sacs and the H-ion concentration determined according to the method already described in this Journal (1915, i, p. 194).

The tubes are noted in which a change in tint from that of the normal blood is evident, and the results are expressed as the c.c. of N/50 HCl or NaOH which must be added to blood to change its H-ion concentration. Thus, the *alkali buffer* is the c.c. of N/50 NaOH which can be added to 2 c.c. of blood without change of the H-ion concentration of the dialysate, and the *acid buffer* the c.c. of N/50 HCl.

The method suffers from the following drawbacks:

1. Such small quantities of acid and alkali are employed.

2. It is often difficult to tell just exactly when a slight difference in tint has been produced.

3. Even with the precautions described above, it is impossible to be sure that the amount of CO_2 in the different samples of blood is the same, which means, of course, that some bloods will, on this account alone, be able to bind more alkali than others.

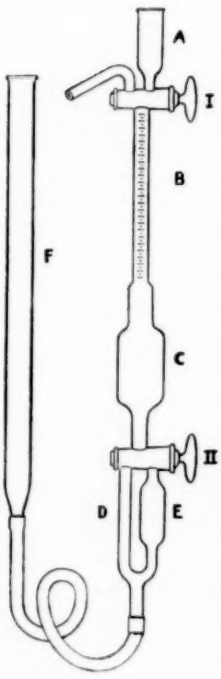
A method based on somewhat the same principle, but which seems to the present writer to be more likely to be of practical value because it meets the above objections, is that suggested by Van Slyke, Stillman and Cullen. Plasma is freed of CO_2 by placing it in a vacuum, and is then mixed with an equal volume of N/50 HCl (or NaOH) and the H-ion concentration determined (this can best be done by the dialysis—sulphonephthalein method of Rowntree, etc.). In the case of normal blood, after such an addition of acid, a practically normal H-ion concentration will be found, whereas in the blood of cases of acidosis it will be found to be very distinctly increased (i.e., P_H lower). The details of this method have not as yet been published, and its principle is described here merely to indicate along what lines progress is likely to be made in the solution of this important problem.

The last mentioned workers have suggested another method, the principle of which is based on the power of the blood to take up carbon dioxide gas. Obviously blood with its reserve of alkali already depleted, as a result of the production of acid substance in the body, will be capable of taking up less CO_2 than normal blood. The apparatus required for this estimation is purchasable* and it is used in the following manner (see illustration†). Blood is centrifuged

*Eimer and Amend, New York, and Arthur H. Thomas Company, Philadelphia.

†The originator of this method has not as yet published full details, but since the apparatus is on the market, it has been deemed advisable to give a general description of its use in this Journal.

and a few c.c. of the plasma shaken in a flask containing 6 per cent CO_2. Alveolar air is suitable for this purpose. The apparatus is meanwhile filled to the top of the graduated tube with mercury by raising the mercury reservoir F, care being taken that D and E are also filled. One c.c. of the CO_2-saturated plasma is then delivered into A and the stopcock I turned so that by cautiously lowering the level of the reservoir F, the plasma runs into B (but no trace of air). The same procedure is repeated with 1 c.c. water, so as to wash in all of the plasma, and finally 0.5 c.c. normal acid (approximately 5% H_2SO_4) is sucked in, after which stopcock I is turned off. The reservoir F is then lowered sufficiently to allow all of the mercury, but none of the blood, to run out of B and C.



As the level of the mercury falls in B and C, the plasma effervesces violently, because it is now exposed to a vacuum. To be certain that all traces of CO_2 have been dislodged from the solution, the apparatus is shaken. To ascertain how much CO_2 has been liberated, stopcock II is now turned so as to bring C and E into communication, and by cautiously lowering the reservoir the fluid in C is allowed to run into the bulb E. Stopcock II is thereafter turned so as to connect C and D, and the reservoir raised so that the mercury runs into C as far as the CO_2, which has collected in the burette will permit it to go. After bringing the level of the mercury in F to correspond to that in the burette, the graduation at which this stands is read. It gives the c.c. of CO_2 liberated from the plasma. Under the above conditions normal plasma binds 75 per cent of its volume of CO_2; therefore, since the total capacity of the pipette is 50 c.c., the mercury should stand at 0.375 c.c. on the burette. In acidosis figures as low as 20 per cent of the normal may be obtained, i.e., 0.1 on the burette.

Finally, to prevent confusion, it should be pointed out that a depression in the reserve alkalinity of the blood can also be determined by determining the percentage of CO_2 in alveolar air by the clinical method already described in this Journal (1916, i, p. 522).

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THE PURITY OF ALCOHOL

By Victor C. Vaughan, M.D., Ann Arbor, Mich.

IN my work with the protein poison, I have found it necessary to give attention to the purity of the alcohol used and I have found the alcohol imported from Germany (Kahlbaum's) not always up to the standard. In fact, I have found it cheaper and safer to take the ordinary alcohol and distill it with quick lime. The following tests may be used in determining the purity of alcohol:

1. The specific gravity is determined by the picknometer.

2. It should be miscible in all proportions without cloudiness with water, ether and chloroform.

3. It should not redden litmus even after four hours' exposure. Kahlbaum's alcohol often reddens litmus in a much shorter time.

4. The evaporation of 50 c.c. should leave no weighable residue. Some samples of Kahlbaum's alcohol gave in this amount as much as 1 mg and some more.

5. A mixture of 10 c.c. of the alcohol, 5 c.c. of water and 1 c.c. of glycerine, allowed to evaporate spontaneously on clean blotting paper, leave no foreign odor when the last trace of the alcohol has disappeared. This is a U. S. P. test and shows the absence of more than a trace of fusel oil.

6. A mixture of 10 c.c. of the alcohol and 0.2 c.c. of two per cent KOH solution is evaporated to 1 c.c. and then treated with an excess of dilute (1:4) sulphuric acid. This should not develop the odor of fusel oil.

7. 10 c.c. of the alcohol is evaporated to 2 c.c. and this is shaken with an equal volume of sulphuric acid. The development of a reddish color shows the presence of amylic alcohol.

8. When 20 c.c. of the alcohol is shaken in a clean glass stoppered bottle with 1 c.c. of silver nitrate, test solution, the mixture should not become more than faintly opalescent or acquire more than a faint brownish tint when exposed to diffuse daylight for six hours. This is a U. S. P. test for organic impurities, amylic alcohol, aldehyde, etc. The imported alcohols are not up to standard at all times by this test.

9. Into a test tube which has been rinsed with the alcohol, pour 5 c.c. of sulphuric acid, then layer the acid with an equal amount of the alcohol. The appearance of a red zone after standing for four hours or longer indicates the presence of molasses alcohol. Neither domestic nor foreign alcohols respond to this test.

10. Pass hydrogen sulphide gas through 20 c.c. of the alcohol for from two to five minutes, then add a few drops of ammonia and allow to stand for four hours. The alcohol should remain colorless. If it becomes yellowish or brownish, the presence of traces of metal, extractives or tannin is indicated. By this test many of the samples of imported alcohol are shown not to be up to standard.

11. To 10 c.c. of the alcohol add 1 c.c. of a solution of potassium permanganate (1-1000). Allow to stand twenty minutes, the development of a yellowish or brownish color indicates the presence of aldehyde. Many of the im-

ported alcohols show this impurity in more than traces. Our redistilled alcohol does not show it.

12. To 5 c.c. of the alcohol add two drops of a one per cent aqueous solution of furfurol, underlay this, kept cold in a stream of water, with 5 c.c. of sulphuric acid. The formation of a colored zone, gradually becoming pink, shows the presence of amylic alcohol. This is an exceedingly delicate test and by making a standard solution of amylic alcohol the proportion can be approximated. Kahlbaum's alcohol, as we have found, often shows appreciable traces of amylic alcohol by this test.

13. To 10 c.c. of the alcohol add ten drops of colorless analin, then three drops of hydrochloric acid. No coloration should develop within five minutes. This is a test for furfurol, which we have not found in either domestic or imported alcohols.

14. Evaporate 100 c.c. of the alcohol to dryness, extract the residue with from 3 c.c. to 5 c.c. of salt solution and inject this intravenously into a guinea-pig of from 200 g. to 300 g. weight. With our redistilled alcohol there is no effect, while with Kahlbaum's the animal is often thrown into convulsions which may terminate fatally. What this residue contains, I have not been able to determine. It is granular and sometimes contains a few needle shaped crystals.

From my experience, I have found it better to use alcohol prepared in my own laboratory from the commercial ninety-five per cent article, by redistillation with caustic lime, than to depend upon the imported product.

WATER BATH SLIDE METHODS FOR COMPLEMENT FIXATION AND BLOOD GROUPING*

By Norman E. Williamson, M.D., Stockton, Calif.

A COMPLEMENT fixation test may be performed on a slide with the small quantity of material according to the following technic:

A rectangular water bath is fitted with thermoregulator at one end. Lateral flanges are placed ½ inch from the top. The bath is filled to within 1 inch of the flanges. On this ledge rest the ends of two glass rods placed across the water bath 2 inches apart. To the glass rods so that it is below them but not long enough to rest on the ledges is tied a rectangular piece of cardboard. There is a narrow slit in this cardboard lengthwise, half way between the rods. The object of this is to maintain the proper amount of moisture on the under side of a slide placed across the rods. If there were no cardboard, too much moisture would be precipitated on the slide and hemolysis of a drop of blood might occur from dilution. If the cardboard is not perforated or slit, the under side of the slide will dry. The size of the slit must be such that a faint haze forms on the under side of the slide which promptly disappears after the slide is removed from the water bath. Over the top of the bath is placed a glass plate except for the end occupied by the heat-regulating apparatus. The open space here is

*From the State Hospital, Stockton, Calif.

closed by a vertical glass plate setting into the water but allowing free interchange of water below it. The plate of glass on top of the bath must not fit too snugly or there will be a loss in a short time of atmospheric dust, and moisture will be precipitated on all objects in the bath. Blotting paper is attached by adhesive to the under side of the glass plate to prevent dripping on the slides. The thermometer is placed on the rods and the heat-regulating apparatus so arranged that the temperature at that point will be 37° C. Constant level is maintained by a filled inverted water bottle with the mouth placed at the level desired.

For the performance of the test very small quantities of standard reagents are prepared. Clean slides, 3 small standard platinum loops, 1 large platinum loop and one straight platinum wire are necessary. The slides are placed on a sheet of paper, each on a number. A loopful of material is taken for the smallest quantity of any of the ingredients of the test and larger quantities of other ingredients are multiples of this smallest amount. For instance, in performing Noguchi's modification of the Wassermann test, it is done as follows: 1 small loop of Noguchi antigen properly diluted is placed on the slide as shown (Fig. 1); 1 small loop of undiluted complement; 2 small loops of patient's serum; 1 large loop of salt solution. These are then mixed with the straight wire and quickly inverted over the water of the water bath across the rods. The time is marked on the paper by the number. Incubate ½ hour at 37° C.

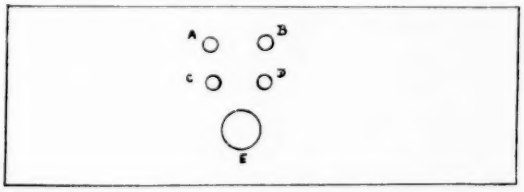

Fig. 1.—A, complement; B, antigen; C, patient's serum; D, patient's serum; E, salt solution.

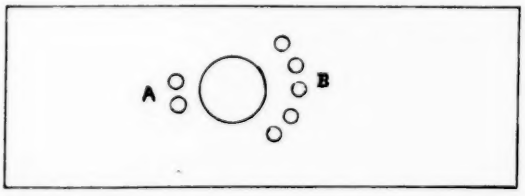

Fig. 2.—A, amboceptor; B, sheep's cells.

Remove slide and add 5 small loops of 10 per cent suspension of sheep's red corpuscles and 2 small loops of amboceptor which is of such a strength that 5 units are contained in 0.5 c.c. (Fig. 2). Mix with a straight platinum wire and replace in water bath. Incubate for 1 hour. Positive specimens are brick red and opaque; negative are clear and brown.

The strength of the complement has been previously determined as follows: One loopful of complement is added to each of 4 loops, 3 loops, 2 loops and 1 loop of salt solution, and one loop of each mixture and also of the undiluted complement is added to 5 loops of the 10 per cent suspension of sheep's corpuscles and 1 loop of amboceptor in the strength given above, and placed in the

water bath for one hour for hemolysis. Twice the strength of the smallest amount of complement giving complete hemolysis is taken for the test.

For Noguchi's test use 2 loops of serum and one loop of complement in case the strength of the complement is such that one unit is 5/100 of a c.c.; 1 loop of Noguchi antigen which has been prepared by adding one part of the methyl alcoholic solution to 7 parts of salt solution; 5 loops of 5 per cent suspension of washed human corpuscles and 2 loops of amboceptor which has been prepared in the ratio of 5 units to 0.2 of a c.c. of salt solution.

Each specimen should be duplicated without antigen to determine anticomplementary action of the serum. The usual positive and negative controls are required. A positive result as above equals ++. If 1 loop of serum gives a positive result it would be ++++. Find the smallest quantity of serum which will give complete inhibition and rate accordingly. By this method the presence of antisheep amboceptor in human blood may be disregarded as excess of amboceptor will have no effect if all the complement is fixed. The same rule is applicable in the use of the human hemolytic system.

Two loops full of serum can be dried on a slide and later dissolved with salt solution and used for the complement fixation test. I have found that the dried blood may require more complement than the fresh blood and I obtained the best results by using sensitized red cells. For this purpose it is well to have an amboceptor which contains comparatively weak agglutinins. To the washed cells in a large quantity of salt solution was added enough amboceptor to make 3 units for the entire quantity of cells. The mixture is kept at 37° for two hours or left in the ice box over night. The cells are then thrown down, the fluid decanted, and fresh salt solution added to make the proper dilution. The complement is titrated with sensitized cells and the best results with dry blood are obtained if twice the amount of complement which will completely hemolyze these cells in 15 minutes, is used. Known positive and negative dry sera must be used for controls.

Kaplan adds his amboceptor to his cells prior to the performance of a Wassermann reaction and his cells are more or less sensitized according to the time and temperature, but he does not make a special effort to have them entirely sensitized as is done in this test. There seems to be no reason why dry blood could not be used for the test tube reaction. 1/10 of a c.c. of serum weighs when dry 10 milligrams; thus 20 milligrams of dry serum could be added to each tube for the Wassermann test after dissolving in salt solution and 8 milligrams for the Noguchi test. Serum can be dried on a clean piece of glass, scraped off, put in a homeopathic phial and sent to the laboratory. It need not be sterile. I have dried some by this method and will test it in a few days.

Since serum is usually readily obtainable fresh the water bath method would not often be required for the diagnosis of syphilis but it may have a distinct value when bacterial emulsions cannot easily be obtained for antigen in the quantity desired for the complement fixation. Test tube methods are easier to perform when control of all conditions is considered.

This water bath slide method was used by me for the purpose of determining the grouping of bloods, using the loops of material as advised by Brem but using no cover-glass or well slide. I use for the test sera of groups 2, 3

and 4 of Moss which have been found by me to be strong in agglutinins. Testing the suspension of corpuscles of the patient with strong sera of groups 2 and 3 will place his group. If the patient happens to have a weak agglutinin for his group, this method is an advantage. For instance, if the corpuscles are agglutinated by group 2, not by group 3 sera, the patient belongs to group 3. If agglutinated by group 3, not by group 2, he belongs to group 2. If agglutinated by both he belongs to group 1. If agglutinated by neither, he belongs to group 4. A further check with the group 4 serum is then used. Since the corpuscles of any group other than 4 may be affected by hemolysin in a patient's serum, it is advisable in using the human hemolytic system for complement fixation test, to use only a group 4 suspension of corpuscles. I obtain them easily as follows: After pouring the serum off the bloods obtained from the patients for Wassermann, I make a suspension of a few of the red cells which I judge to be approximately 5 per cent and see if they will be agglutinated by a group 4 serum I keep in stock. If they are not the blood belongs to group 4. I then shake out of the clot by the aid of a little salt solution some red cells, wash them in the usual manner, using them for the fixation test. I have found that on an average without getting either fibrin or leucocytes I can shake out 3/4 c.c. of red cells from my bloods by this method. I use such cells for the immunization of rabbits after having determined that the Wassermann in that case is negative and the history of the patient negative. Such cells can be mixed safely only after all the serum has been removed. I killed two rabbits by a failure to observe this precaution as agglutination of corpuscles may occur if the blood belongs to different groups. I find this much the easiest method to get normal cells.

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EDITORIALS

*Medicine as a Career**

STUDENTS of Medicine: You have chosen your life work. You have elected to devote your time and energy to the science and art of medicine. It is hoped that you fully realize the importance of this decision and that you have not come here without adequate deliberation and comprehension of the heavy tasks you have assumed in taking this step. In view of the possibility that some of you have made a mistake, I have decided to spend this hour in presenting to you a few of the duties and obligations which you are assuming, and if there be among you those who feel that the burdens to be borne are too heavy and the personal gain too light, let such not hesitate to stop and turn back on the threshold. Medicine needs recruits, but it desires and will accept only those who after severe tests, it deems worthy. I am aware of the fact that the words of the experienced fall lightly upon the ears of the inexperienced, but one who has served in the ranks for nearly forty years offers you advice. I wish to say that the fatality among medical students is great. In the past ten years, less than sixty per cent of those who have entered this school have succeeded in winning its diploma and of those who have gained this distinction, not

*Address at the opening of the University of Michigan Medical School, Oct. 3, 1916.

all have fulfilled the confidence imposed in them by the faculty. It is not my expectation that you will do better than preceding classes.

Medicine embraces all facts which may be utilized in the prevention or alleviation of disease. Its chief contributory sciences are physics, chemistry and biology. It is for this reason that a knowledge of the fundamental principles and facts of these basic sciences is required for admission to the better medical schools. Some of you will fail because your training in these sciences has been inadequate. Teachers in the medical school cannot take the time nor can they hold back better trained students to instruct those who are deficient. By the end of the first year most of these unfortunates are asked to withdraw.

With the best possible preparation the medical student finds his daily task, quite as much as the strong can carry and altogether too heavy for the weakling. There has been some discussion among medical educators concerning the curriculum, some contending that it is too heavy for the average student. This depends upon what is meant by the "average student." If the standard set in college work is applied, I am of the opinion that medicine does not want such "average students." I am convinced that a strong student, of a high average, can carry the medical work as now imposed and that the imposition of a heavy task succeeds in weeding out the unfit and is therefore desirable. We do not develop muscles by lifting feather weights, nor do we strengthen brain activity without earnest effort. The aim of medical education is to develop strong men and in order to do so, difficult tasks must be imposed in the training.

A strong intellect is not enough to insure success to the medical student. Intellect must be backed by industry, otherwise it is of but little value. For lack of industry many medical students fall by the wayside. After forty years as a teacher in this school, I am of the opinion that lack of proper application to the work is the most potent cause of failure among the students. In his collegiate course the work has been light, easily done. He has had a good record, but has failed to establish habits of study. Some allurement causes him to neglect his tasks for a day and then for a week. Soon, he finds himself quite in the rear. His bluff at recitation does not go. His teachers question his intellectual strength and honesty. He becomes a derelict and must be removed for his own and others' good.

A third essential to success in medicine is integrity. When endowed with a high degree of intelligence, supported by the greatest industry but without integrity the medical man is likely to prove a disgrace to his profession and a menace to the community in which he lives. That integrity has been regarded as an essential qualification of the practitioner of medicine from the earliest times is shown by the exaction of the Hippocratic oath supposed to have been formulated by the father of the profession. The medical man must be honest with himself, his patients and the public. For personal gain he must not pretend to greater knowledge or skill than he possesses. Professional ethics insist that in the announcement of his purpose to serve the community he must restrict himself to the simplest statement. The public has long ridiculed the restrictions which the medical profession has attempted, with more or less success, to impose upon its own members, but that the public is now reaching a point where it appreciates the righteousness of medical ethics is shown by recent

legislation forbidding false and exaggerated advertisements. The first thing for the honest man in becoming a physician to do is to secure the best possible preparation. To enter upon the practice of medicine or to continue in it without adequate preparation is a crime—a moral, if not a statutory one. The public has come to this view and there is no other profession, admission to which is so strictly guarded as that of medicine. State laws set the standards of admission to medical schools and State Licensing Boards test medical graduates. The best intentions do not supply the deficiencies due to lack of knowledge and skill. It certainly can be said that in the practice of medicine, knowledge is a virtue and ignorance a crime. Recognizing the fact that no man, however great his intelligence and untiring his industry, can be skilled in all branches of the healing art, individuals select specialties in which they strive to make themselves experts and these so group themselves that each patient may have the advice of an expert. The wisdom of this procedure and its advantages to both practitioner and patient must be evident to all. Each individual in such a group must know his specialty and must keep in touch with its progress. Medicine is a progressive science. Each year adds to its effectiveness. Discoveries in physics, chemistry and biology find practical application in the prevention or cure of disease. It follows that the efficient medical man must continue to be a student so long as he remains an active member of the profession. In medicine there are no "papal bulls," no "ipse dixits" and even "precedent" is shown but scant respect. It is best compared to a living plant constantly drawing sustenance from soil and air, dropping its withered leaves and branches, ever putting forth buds and blossoms and bearing each season better fruit. One who is not capable of sustained effort, should seek some other calling in life. Occasionally I meet with men who are still living professionally in their undergraduate days, reading the same old books, and writing the same old prescriptions, both blind and deaf to the changed environment. Fortunately the more intelligent of the public easily recognize these fossils and appraise them at their true worth. They are interesting as relics of the past but worthless in the present.

From the time of Hippocrates to the present, wise men in the profession have always advocated amity among its members and I must say after many years of personal experience that there is no other high professional ideal so difficult in realization, but I am proud to add that there never has been a time when the promise of the realization of this ideal has been so great as at present. In this matter medical men have learned much from the commercial world in which the value of cooperation has been so abundantly demonstrated. The efficiency of the individual has been increased and the value of the product has been improved. Much regret has been expressed concerning what is called the passing or the elimination of the old-time family physician or general practitioner. In the slow development of scientific medicine he served his fellow-men, often with the greatest devotion and self-sacrifice. The history of epidemics shows him to have been often worthy of the highest honor. He has faithfully served his fellow-men in times of dire distress. Occasionally he has made contributions of the greatest value to science. In the record of the slow progress of man from the marshes of ignorance and superstition to the uplands of knowledge and

science he bears a conspicuous and honorable place, but in the practice of modern medicine his part is a subordinate one.

In any community in which several physicians are singly doing a general practice, cooperation, with the development into skilled specialists, results in individual efficiency among the medical men and better service to their clientele. With a properly equipped hospital at their service, a group of village physicians may give their patients the same scientific and effective treatment that they can secure in larger medical centers. I have no sympathy with the contention that our rural population demands cheaply educated physicians. With trolley cars and automobiles there are but few in need of medical aid who are so located that a good physician may not soon reach them, or what is better, that they cannot soon be transported to a hospital. A friend who has long practiced in a small Montana city recently told me that twenty and more years ago, his ride sometimes carried him one hundred and twenty-five miles from home. One such visit to a case of pneumonia is unsatisfactory to the doctor and of but little benefit to the sick man. Now, the automobile brings the patient to a well equipped hospital where several physicians may daily consult concerning the case and trained nurses be constantly in attendance.

When I was in medical practice, like the milkman, I made my daily rounds seeing cases of scarlet fever, diphtheria, sometimes smallpox, pneumonia, various nervous diseases, attending cases of labor and in short, I was a general practitioner. Like others of the kind I did the best I could for all and I am able to say with some pride that in no case did I carry infection from house to house. However, in order to avoid this I often had to change my clothing and disinfect my person many times in one day. This kind of practice is still largely in vogue, but it is gradually being displaced by hospital treatment in which specialists direct and trained nurses administer. The greatest need of practical medicine today is more and better equipped hospitals. With these the specialist and the skilled nurse will multiply and improve. Such hospitals should be supplied with thoroughly equipped and competently manned diagnostic laboratories which should serve not only curative but preventive medicine. Water and milk supplies should be examined daily and visiting nurses under the direction of a competent health officer should constantly patrol the community. Adjunct dispensaries which should serve as schools of instruction in baby feeding and care, child welfare, home sanitation and in everything pertaining to healthy living should supplement the community hospitals. When in addition to these agencies the people generally can be educated to see the benefits that would follow the periodic thorough examination of all in order to detect the first departure from the normal, then medicine will be able to render its highest service to mankind.

It must be evident that if these hopes are to materialize the practice of medicine must become more and more a State function. That the tendency is in this direction and that this should be encouraged for the public good are not matters of doubt in my mind. Only a few years ago some of the most eminent men in the profession combatted earnestly State support of medical education. They claimed that the State had no right to establish and maintain medical schools and that such aid was not fair in competition with the proprietary schools, which at that time educated more than ninety per cent of the annual recruits to the profession. Now, no one questions either the right or the duty of the State to

establish and support medical schools, while the proprietary schools, having proven wholly inadequate and inefficient, have practically ceased to exist. Even the man in the street sees the advantages that have resulted from these changes. To state that a medical school is a proprietary one, in the sense generally understood by that term, immediately condemns it with intelligent men. Courts from the lowest to the highest in the land have uniformly held that the State has the right to maintain its own medical school, also to pass upon the merits of other schools both within and without its borders, to set up standards of medical education, to define the requirements of admission to medical schools, to submit those who wish to practice within its borders to certain intellectual and moral tests in order to pass upon their professional fitness and to revoke the licenses of the unworthy. The Federal Government has its public health service which passes upon immigrants, controls national quarantine, maintains a research laboratory, supervises the manufacture and sale of vaccines and antitoxins, and stands ready to aid any State in combatting epidemics. Each State has its Board of Health, the powers, functions and efficiency of which vary widely. Our great municipalities have their boards of health and commissioners of health which for the most part are efficient, but in some instances are parts of a political machine. Our smaller cities and rural communities have their boards and health officers, which with some notable exceptions, fortunately in increasing members, are cheap, ignorant and inefficient.

By means of these organizations, imperfect as many of them are, the death rate in the registered area of the United States has been reduced in the past thirty years from twenty to fourteen per thousand, the average life has been increased more than ten years, and the mortality from tuberculosis and other infectious diseases has been reduced about fifty per cent. On account of the greater efficiency of the health service in our larger cities, the reduction in the death rate has been more marked in these than in smaller cities and rural communities. The greatest reduction in mortality has been secured in our cities of one hundred thousand or more. Our metropolis, New York, has a municipal health service which is second to none in the world. It supports a research laboratory in which the highest grade of scientific investigation is done, diagnostic laboratories in which diphtheria cultures, suspected sputum, blood examination and other tests essential to scientific medicine are made and laboratories in which water and food supplies are carefully guarded. It has a corps of expert diagnosticians ready to aid the practitioner in all suspected cases, free of charge to either the medical man or the patient. It provides medical school inspectors who detect infection in its earliest stages, excellent hospitals in which the sick have the best care and treatment and nurses who patrol the tenements and other homes of the poor and give instruction in sanitation. It examines cooks and waiters to see that none of these may distribute typhoid fever, tuberculosis, syphilis or other infections. It inspects meat markets, bakeries, milk stations and other places of food supply and has the authority to close these when unsanitary conditions are found.

The last legislature of Michigan made an appropriation of one hundred thousand dollars and directed the State Board of Health to expend it in attempts to restrict tuberculosis. Several thousand citizens have already been examined free of charge in order that this disease may be detected in its early stages when it is amenable to hygienic treatment. These people are not only examined, but

those found infected are instructed how to live in order to avert the progress of the disease.

I have chosen to bring these matters before you in order to impress upon you the relation which the profession, which you have selected, bears to the public. Even the physician who devotes himself wholly to what is known as private practice does not escape his duties to the public. He is morally bound not only to do his full duty to the individual, who employs him, but to protect the community. You have chosen to come to a school supported by the State. Michigan practically gives you your education. Why does it do this and what does it demand of you in return for this great gift? It expects that you possess intelligence, for without this, the gift is valueless; that you manifest industry both during and after your student life, for without this, you bury your talent; that in all your actions, both professional and nonprofessional, you show the most sincere integrity, for without this, you become a menace to your benefactor. The State has selected this faculty to ascertain to what extent each of you possesses these essential qualifications and I can assure you that those found wanting will not find their way into the profession through these doors. To those, who prove worthy, every reasonable encouragement and proper assistance will be given.

I am sometimes asked what financial reward can the medical man reasonably expect? This is a proper question and I am ready to give it my answer. In the first place a medical education, even with the relatively small tuition one pays in a State University, is the most expensive professional education, both in time and money, both to the State and to the student.

The laboratory expenses of the medical student are higher than those in any other school. Where other students buy books, he buys not only more expensive books, but he must also purchase a microscope, blood counter, and other expensive instruments. After graduation most medical students spend from one to three years in hospital work and at least one of these promises soon to become obligatory on all. When he begins practice the medical man must have a respectable office and a well equipped laboratory. He must continue to buy expensive books for the average medical book is out of date almost as soon as it leaves the press, so rapid has been the advance in scientific medicine in the past thirty years. He cannot do without the best professional journals and being a member of a learned profession he is ashamed to be ignorant of the best general literature. In his consulting room, his visits to the homes of his patient and in his association with his fellows he must be neatly, though he need not be expensively, dressed. He must supply himself with means for quick and comfortable travel. Without going into further particulars I may say that by the time he is ready to begin his professional work, the most economical medical man has already made an investment of from ten to twenty thousand dollars, counting his actual expenses, allowing a fair amount for his time and calculating the interest on these amounts, and when he begins, he must have the wherewithal to make his work successful. No medical man can neglect the financial side of his life's work. Without an adequate income he cannot reach a high degree of efficiency in his work. However, the medical man who is imbued with the right spirit will use his financial gains largely in increasing his professional efficiency. After setting aside enough for the fair support of himself and those dependent upon him, he will devote the surplus— and there must be a surplus if he is to be

successful—to better equipment, both physically and mentally. It has been my observation that the more intelligent laity respects the physician, who endeavors to keep himself well posted and well equipped in his professional work. Medical men who attend their local, State and national societies are as a rule successful financially while those who think that they cannot leave their work even for self-improvement, have a hard time in making ends meet. One who wishes to accumulate a fortune or to become wealthy as that term is now understood, should choose some other calling. I know of no one who has placed himself in this class by the reputable practice of medicine. Some medical men have made riches by fortunate investments, but this is an exception. Some marry wealth, but this is usually fatal to professional efficiency. I know of but one man who has demonstrated his ability by winning the highest distinctions in the profession notwithstanding the fact that he married a wealthy woman. While on this point, I may say that practice coming from the ultra-rich is not to be coveted. They are exacting in their demands and poor pay. They object to ordinary bills and cry out that they are being sandbagged. As I write this, I have before me such a letter from a millionaire. He admits that he selected the medical man on account of his recognized skill, that he knew what the charges would be before the services were closed and that he did not object at that time, because he was afraid that the medical man would desert him, but when payment was demanded, he claimed that he was being sandbagged because he was known to be rich. The ultra rich are familiar with the use of the sandbag in extorting money from others and they see its phantom in even the most moderate bills presented them.

The medical practitioner endowed with intelligence, fortified with industry and with his every action controlled by strict integrity is sure to make a decent living, care for himself and family in comfort and he need not sleep in a pauper's grave. He is not compelled to sacrifice his self-respect to expediency. His calling is quite as independent as any other. He can choose his own friends, church and political affiliation. The man who is sick with pneumonia, or has an inflamed appendix does not consult the society columns, the church directory nor the polling lists when he selects his medical attendant. He prefers the man who is likely to render him the best service and the intelligent public in the long run and on the whole judges wisely. There never has been a time when individual worth among medical practitioners was more correctly evaluated and I may add, more highly estimated, than the present. Medicine has cast off the veil of mystery which once covered her face and walks among men, uncovered and unashamed. The days of "divine healers," Indian medicine fakirs and of Mrs. Winslow and Lydia Pinkham are passing away. Some may say that these statements are contradicted by the wide prevalence of christian science, osteopathy and other cults. These are only the vagaries which have taken form in the delirium racked brain of a fast dying superstition. Did our Government select any of these agencies in its successful combat with yellow fever in Cuba or on the Canal Zone? Has it relied upon them to keep Asiatic cholera or the plague out of this country? Did it send christian scientists or osteopaths to stay the epidemic threatened by the Dayton floods? Are these cults now busy healing the wounds and adjusting the dislocated bones so abundant on European battlefields? Our Lady of Lourdes and Ste Anne Beauprie are apparently not on duty at a time when shell-

torn and flame-tortured humanity is in greatest need of their much extolled, miraculous powers of healing. The genuine worth of scientific medicine has never been so thoroughly tested as in the present war. Amid unprecedented difficulties, in the camps where millions are congregated, in the quick transportation of corps after corps, in the trenches and even among the prisoners of war, always cared for grudgingly and reluctantly, everywhere, preventive medicine has successfully met her old foes, typhoid fever, dysentery, cholera, tetanus, and other epidemics, which in former wars have usually been the most destructive factors in the midst of contending armies, and have often decided battles and determined the fate of nations. Decisive victories have not yet followed the flags of the central or the allied armies, but in all, the Red Cross signalizes the most triumphant achievement of man. International laws have been torn into shreds and become mere scraps of paper, moral and religious precepts and codes have been supplanted by brutalities never practiced by primitive man and the foundations of civilization have seemed to be on the point of disruption and final collapse, but the spirit and ideals of scientific medicine remain unsullied and a new world in which these shall dominate will be created.

Medicine offers a number and variety of special activities to those who choose it as a career. First, there is the grand division into preventive and curative. The former is a product of the Nineteenth Century, the latter as old as the records of man. The oldest and still a widely dominant theory, as to the cause of disease is that it is an infliction laid upon man by some supernatural being. Primitive man, which term once embraced all, and in this particular, still includes many, probably a majority, even among the most highly cultured nations, believed in the existence of powerful spirits, who measured out good and ill to individuals as their own will might indicate. The religion of such believers consisted and still consists in attempts to propitiate these powerful, or one omnipotent, spirit. They built and still build altars of sacrifice and temples of devotion in which they proclaim their own weakness and implore divine protection and guidance. They still beseech a supreme ruler to shower blessings upon themselves and curses upon their enemies. In the hands of the Jehovah of the Jews disease was a scourge for the punishment of those who merited his displeasure. In the adoption and modification of the Hebrew religion by the Christian world, the idea of a God of wrath was adopted, and still prevails. Even today in battle scarred Europe, the same God is invoked and his aid asked in each contending army. With this inborn superstition transmitted through countless generations, scientific medicine has had to contend. The combat has extended through centuries as is shown by the earliest records of human achievement. The first signal victory was won when Jenner robbed smallpox of its horrors by the discovery of vaccination and success was assured by the labors of Louis Pasteur who marked the way by which each infection may be identified, controlled and abated.

An enlightened public is beginning to recognize that many diseases, especially the infections, are preventable and the medical profession is being called upon to plan and direct this work. Many of the smaller cities and some rural communities are providing for full time health commissioners and the demand is greater than the supply. This and other universities are conducting courses specially suitable for public health officials. I am sure that some of you will

select this field for the development of your life work. In it there is abundant opportunity to do credit to yourself while you serve the highest interests of your fellow-man. The labors of Reed and his colleagues demonstrated the agencies by which yellow fever is spread and Gorgas and his helpers freed Cuba from this disease and won a greater triumph in the Canal Zone. Laveran and Ross did even a greater service in showing how the world may free itself from malaria, which in all times has held some of the fairest and most fruitful lands under its curse. Preventive medicine is now capable of opening up the tropics as suitable habitations for civilized man, of removing the stigma of being the "home and nursery of disease" from the fertile valleys of the Nile, and of returning to cultivation the banks of the Euphrates and the Tigris on which the cradle of civilization was rocked. I cannot believe that coming generations will be so insane as not to use this most potent agent in reclaiming the marsh, the wilderness and the barrens, and converting them into fields, rich in agricultural products and abundant in happy homes. Man's destiny is in his own hands and he may make of this earth a heaven of peace, plenty and prosperity, or he may mar it into a hell of strife, rapine and murder. In knowledge he has advanced to a position in which he becomes a co-worker with the Creator, and he must bear the responsibilities which such power imposes. In the struggle between good and evil, knowledge and ignorance, science and superstition, medicine has, and must continue, to lead the way, and you as its standard bearers must serve your day and generation with intelligence, industry and integrity. I do not mean that you are to do your work, always conscious of the burden of duty. With all its imperfections this life is worth living and its highest joys lie in its contests. The man who does not get real pleasure out of his work remains a poor workman and his products do not find ready sale in the market. Even the bitterest disappointment, when you have done your best, often becomes a beacon light warning you of the rocks and leading you into a safe harbor.

It must not be inferred from the great stress that I have placed upon preventive medicine that the curative art is not equally worthy. Moreover, cure is not going to be replaced wholly by prevention. Disease and accident will continue so long as man reproduces his kind. The history of this, the older, branch of medicine, is that of man's efforts to relieve the distress and to minister to the needs of his fellow man. Born in ignorance, nourished on superstition, clothed with mysterious rites and ceremonies, medicine has had a hard task to free itself from hereditary and environmental influences. Attempts to break away from these adverse and retarding conditions have marked the highest efforts of the race. During nearly every century since recorded history began, there have been some superior men, intelligent and far-seeing above the masses, who have contributed something to science. Such were Hippocrates, Galen, Pare, Servetus, Harvey and others whom we now delight to honor as contributors to knowledge and benefactors to the race. The discoveries, by empirical methods, of the specific effects of Peruvian bark in malaria and of mercury in syphilis did much to improve the condition of life and to enlarge the field of human endeavor. Since the scientific era began, the marvelous virtue of antitoxin in diphtheria; its great value in tetanus; the relief of cretinism by thyroid feeding; the action of thymol in hookworm disease; the benefit of salvarsan in the treatment of syphilis; the Pasteur treatment of hydrophobia; the prevention and cure of berri-

berri by nutritional regulation, mark some of the most evident achievements in curative medicine. For diagnostic and prognostic purposes the medicine man of primitive peoples consulted oracles, watched the peristaltic movements of the intestines of animals offered in sacrifice, or read the fate of his patient in the positions of the stars. The physician of today employs the discoveries in physics, chemistry and biology for these purposes. The physician of fifty years ago was compelled to rely largely upon the study and interpretation of symptoms in which the best became highly proficient; today he supplements these studies with the microscope, Roentgen ray, test tube, and other instruments of scientific precision. Then, his conclusions were drawn largely from guesses, now they are founded upon exact and positive knowledge. A large part of your undergraduate education will consist in familiarizing yourselves with the use and application of instruments of precision for diagnostic purposes. Each year brings forth advances in the fundamental sciences and medicine is ever ready to utilize such discoveries as may be of service in the prevention or cure of disease. It has been demonstrated that the physiological action and therapeutical effects of a chemical compound can be modified by changes in its molecular structure. The genius of Ehrlich produced salvarsan and its later substitutes in accordance with this principle and the possibility of finding curative agents in other diseases by similar investigations is now occupying the time and energy of many laboratory students. While the achievements of preventive medicine have greatly reduced the numbers of those infected, medicine is not neglecting its curative agents and we can confidently expect great results in this direction.

The advance of modern surgery has been marvelous. No greater gifts has science brought to suffering man than surgical anesthesia, the discovery of which American medicine can justly boast, and aseptic surgery, made possible by the fundamental work of Pasteur and given practical application through the genius of Lister. These discoveries enable the surgeon to penetrate every part of the body and remove diseased tissue, repair injuries, extract foreign bodies and restore the individual to health and efficiency while he sleeps wholly unconscious of the operation. Plastic surgery has become a fine art and the successful transplantation of tissue is being practiced in the base hospitals of Europe, where the brutalities of man are being ameliorated by skillful operation. The possibility of not only preserving but of growing animal tissue *in vitro* has been demonstrated and has developed a reasonable hope that the surgeon of the future may do still greater miracles.

The development of medicine must be preceded by scientific discovery, because medicine consists in the application of these discoveries. It follows that the highest duty of the medical man is to make contributions to scientific advances. In the past, medical men have made an honorable record in this direction and there is no branch of science to which they have not brought valuable contributions. Even at the present, the open field of knowledge is of small dimensions, while on every side extends the boundless wilderness of ignorance. It has been a great privilege and a joy to have lived at a time when my chosen profession has been so rapidly moving forward and to have met face to face so many of its leaders. It has been my fortunate lot to work in the laboratory of that great German, Koch, to have listened to the words of that great English-

man, Lister, to have enjoyed the friendship of that great Russian, Metchnikoff, and to have looked into the kindly face of the greatest man of the generation, if greatness be measured by good done one's race, that Frenchman, Pasteur. May some spark of the genius which led these men to great accomplishments descend upon and abide in you.

—*V. C. V.*